Chromosoma, Bd. 6, S. 1—32 (1953).

Aus dem Genetischen Institut der Universität Helsinki.

VERSUCH EINER KERNANALYSE BEI DEN SOMATISCHEN HODENZELLEN DER APHODIUS-ARTEN (COLEOPTERA, SCARABAEIDAE).

Von

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Mit 8 Textabbildungen.

(Eingegangen am 29. April 1953.)

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I. Einleitung.

Die Klarlegung der Chromosomenverhältnisse in den somatischen Zellen wird besonders bei den mehr differenzierten Geweben oft dadurch erschwert, daß die Zellen ihr mitotisches Teilungsvermögen verloren haben und in dem Ruhezustand verharren. Die Chromosomenzahl kann dessen ungeachtet auch in solchen Zellen klargelegt werden, wenn nur der Chromosomensatz eine bekannte Zahl in den Ruhekernen sichtbare und identifizierbare Körper: Chromosomen, Chromosomenabschnitte oder sogar Nukleolen, enthält. Vor allem kommen die heterochromatischen Geschlechtschromosomen dabei in Frage, wie z. B. bei *Gerris* (GEITLER 1937). Enthält der Kern aber sehr viele allocyclische Chromosomenabschnitte, so kann die Deutung der interphasischen Chromatinfiguren schwierig oder sogar unmöglich werden, und zwar besonders wenn die Körperchen eine Neigung haben, zu größeren Sammelchromocentren zusammenzutreten. In solchen Fällen kann z. B. die Polyploidiestufe

nur mittels der Kerngröße und der Heterochromatinmenge kalkuliert werden.

Auf derartige, in den Hoden befindliche Somakerne wurde meine Aufmerksamkeit gelenkt, als ich die Chromosomenverhältnisse der Spermatogenese bei einigen Vertretern der Gattung *Aphodius* studierte. Bei diesen heterochromatinreichen Käfern verläuft die Prophase der Spermatocyte I wegen der Chromocentrenbildung und des kräftigen Wachstums der Zelle abweichend (VIRKKI 1951). Im vorliegenden Zusammenhang wird nun versucht, den Kernbau einiger somatischen Hodenzellen der *Aphodius*-Arten im Lichte der bisherigen und neuer Beobachtungen über die Cytologie dieser Tiere zu analysieren.

II. Material und Methode.

Das Material umfaßt insgesamt 15 *Aphodius*-Arten, dieselben wie in meiner früheren Untersuchung (VIRKKI 1951), auf die hier für die das Material betreffenden näheren Daten (Einsammlungsorte und -zeiten, Präparation usw.) verwiesen sei. Hier ist nur zu erwähnen, daß ausschließlich Schnittpräparate zur Untersuchung kamen.

In der vorliegenden Arbeit haben sich 2 Arten: *A. foetens* FABR. und *A. fimetarius* L., aus der Untergattung *Aphodius* s. str. MULSANT, wegen der Deutlichkeit ihrer Kernstrukturen von den übrigen abgehoben und haben darum eine zentrale Stellung in den Untersuchungen erlangt.

Alle Zeichnungen sind unter Anwendung des Ölimmersionsobjektivs 100mal und des Okulars 25mal mit Hilfe des Abbe-Zeichenapparates (alles Zeiß) auf der Höhe des Arbeitstisches ausgeführt worden. Bei der Druckwiedergabe der Abbildungen hat eine $^1/_2$malige Verkleinerung stattgefunden, wodurch die endgültige Vergrößerung 2225fach ist.

Photographiert wurde mit dem Mikrophotographieapparat Zeiß Ikon unter Benutzung des Okulars Photo 18mal von Winkel-Zeiß und des Objektivs 90mal (Apochromat, n. A. 1,3) von Zeiß. Alle Aufnahmen sind unretuschiert.

III. Beobachtungen.

In der Familiengruppe *Lamellicornia* sind die Hoden paarig und dazu „zusammengesetzt-büschelförmig" (MEIXNER 1936), d. h. von mehreren Teiltestikeln gebildet. Die letztgenannten sind in ihrem Bau radiärsymmetrisch in bezug auf diejenige Achse, die man sich in der Richtung des austretenden Vas deferens den Testikel durchlaufen denkt. Ihre äußere Form ist bei den Aphodinen kugelig; von den Besonderheiten des inneren Baues sei der Mangel von Septen wenigstens bei den Imagines erwähnt. Die Hoden enthalten sechserlei Somazellen: 1. die Zellen der Außenmembran, 2. die Wandzellen des Vas deferens, 3. die großen Sekretzellen des kapselartigen inneren Teiles des Vas deferens, 4. die Cystenzellen, 5. die Zellen der peritonealen Hülle und 6. die hier und da zwischen den Meiosecysten vorkommenden Somazellen von wechselnder Größe, die ich hier in Ermangelung eines genaueren Namens *Interstitialzellen* nenne.

Für dieses somatische Gerüst des Hodens ist charakteristisch, daß die Kerne aller Zellarten deutliche und wahrscheinlich sehr beständige Chromocentren enthalten. Im folgenden werden hauptsächlich die Interstitialzellen behandelt, weil die Kernstruktur bei ihnen besonders interessant zu sein scheint.

1. Die Interstitialzellen.

Bei den *Aphodius*-Arten setzt die Spermatogenese sehr früh im Frühjahr ein, vielleicht schon ehe die Tiere ihre Überwinterungsstellen verlassen haben. So enthält auch mein Material lediglich Individuen, bei denen die Spermatogenese schon weit fortgeschritten ist. Die lange andauernde diplotäne Wachstumsphase ist gewöhnlich das vorherrschende Stadium in den Hoden. Zu dieser Zeit begegnet man den Interstitialzellen überall zwischen den Meiosecysten, am häufigsten jedoch beim kapselartigen Beginn des Vas deferens. Beim ersten Blick scheinen sie unregelmäßig zerstreut zu sein, doch bilden sie vielleicht tatsächlich ein derbes Gerüst im Hoden. Das Cytoplasma ist in diesen Zellen sogar bei Eisenhämatoxylinfärbung ziemlich klar, obschon es einige intensiv gefärbte Einschlüsse enthalten kann (Abb. 2b). Ohne jeden Zweifel haben die Interstitialzellen etwas mit der abweichenden Stoffwechseltätigkeit zu tun, die den Riesenwuchs der Spermatocyten verursacht (s. VIRKKI l.c.). Dafür spricht auch die Kleinheit und Seltenheit der betreffenden Zellen bei den Scarabäidenarten mit normaler Spermatogenese. Es ist möglich, daß die Interstitialzellen ontogenetisch den Septenwandzellen nahe stehen oder sogar deren Abkömmlinge sind. Die oft feststellbare platte Form kann ein Zeichen von solchem Ursprung sein.

Die Kerne der Interstitialzellen variieren in der Form von kugeligen und ellipsoidischen bis zu den unregelmäßigsten platten, buckeligen und lappigen Formen. Die Größe der Zellen und dabei auch der Kerne variiert gleichfalls beträchtlich. Auch in ein und demselben Hoden können die Kerne von sehr verschiedener Größe sein. Eine so beträchtliche Größenvariation berechtigt uns zu dem Verdacht, daß die Interstitialzellen außer diploid auch verschiedengradig polyploid sein können, um so mehr, als wir ja wissen, daß die somatische Polyploidie im Insektenhoden keine Seltenheit ist (s. z. B. GEITLER 1939, 1943; OKSALA 1939).

A. Form und Zahl der Chromocentren.

Die Kerne sehen besonders in gutgefärbten Feulgen-Präparaten sehr auffallend aus. Alle enthalten eine Menge von gleichmäßig über die Kernmembran zerstreuten, stark feulgenpositiven Chromocentren. Die Chromocentren der kleineren Kerne scheinen im allgemeinen kleiner zu sein als die der größeren. Von dieser Regel gibt es allerdings Ausnahmen. So kann man z. B. in einem kleinen Kern ein oder mehrere ungewöhnlich

große Chromocentren sehen (vgl. Abb. 1b und c, Abb. 2a). Diese sind zweifelsohne durch Verschmelzung mehrerer kleiner entstanden, denn die Chromocentrenzahl ist in solchen Kernen kleiner als gewöhnlich. Es gibt auch Formunterschiede, diese sind jedoch nach meinen Beobachtungen von der Kerngröße unabhängig. Ungefähr 60—70% der Kerne enthalten sternförmige Chromocentren, bei denen das kompakte, bisweilen zart vakuolisierte Zentrum lockerere, meistens der Kern-

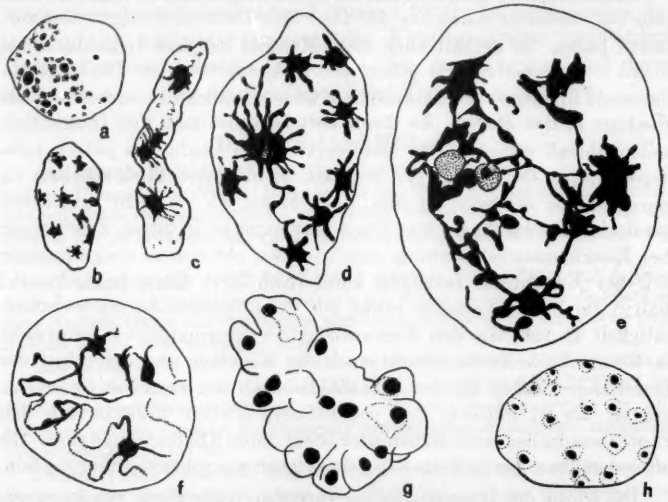

Abb. 1a—h. Kerne von Interstitialzellen von *Aphodius fimetarius* (a, b, e, f) und *A. foetens* (c, d, g, h). In a, e und f ist der Kerninhalt nur teilweise, in den übrigen Bildern vollständig eingezeichnet; Nukleolen in e punktiert. San Felice; b und e: Hämatoxylin; c, d und h: Feulgen, bei den übrigen dazu Lichtgrün.

membran dicht anliegende Äste entsendet (Abb. 1b—e, Abb. 3c). Ihr Aussehen entspricht in großen Zügen den früher in Somazellen gefundenen sternförmigen Chromocentren (vgl. z. B. HEITZ 1932, 1933a, b; MAKINO 1940; GEITLER 1948a, b; MONTALENTI 1949) oder auch den für die *Aphodius*-Arten charakteristischen frühdiplotänen Chromocentren. Die Zahl der Äste variiert je nach der Größe des Chromocentrums und erscheint zudem in größeren Kernen durchschnittlich einigermaßen höher als in kleineren. Ihre genauere Ermittlung hat sich jedoch als unmöglich erwiesen.

Alle sternförmigen Chromocentren sind indessen keineswegs untereinander gleich. Es gibt Chromocentren, deren Äste nahe beim Zentrum nur schwach oder gar nicht gefärbt sind. Das Zentrum scheint dann von einem hellen Ring umgeben zu sein, aus dem die sichtbaren Abschnitte

der Äste ausstrahlen (Abb. 2a, rechts). Auch die Länge und Dicke der Äste variieren, letztere vielleicht durch das Zusammentreten mehrerer einzelner Äste. Eine perlschnurartige Struktur ist oft feststellbar. In extremen Fällen konkurrieren die chromomerenartigen Verdickungen der Äste mit dem eigentlichen Zentrum, so daß der Kern mit kleinen Chromocentren überfüllt zu sein scheint (Abb. 1a; Abb. 3b).

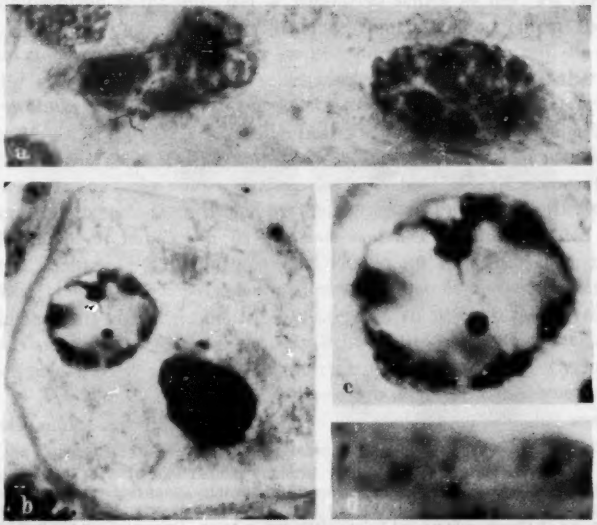

Abb. 2a—d. Interstitialzellen. a *Aphodius foetens*, 2 Kerne von gleicher Größe, in dem rechten etwa 10 kleinere, in dem linken nur 3, dafür aber um so größere Chromocentren. San Felice; Feulgen + Lichtgrün. b—d *A. fimetarius*. b Die größte gefundene Interstitialzelle. c Kern stärker vergrößert. Man beachte den runden, zwischen 2 Chromocentren „gespannten" Nukleolus und den großen Einschluß im Cytoplasma. d Kern einer kleineren Interstitialzelle mit unverästelten Chromocentren (vgl. b oben links). (b—d San Felice; Eisenhämatoxylin). a, c, d 1430mal, b 715mal.

Seltener als die sternförmigen sind die völlig unverästelten Chromocentren. Etwa 30% der Kerne enthalten derartige kugelige Chromatinkörperchen. Ein heller Hof um das Zentrum kommt häufig vor und ist auch bei den Feulgen-Präparaten feststellbar; denn das übrige Kernplasma hat die Neigung, sich durch Einwirkung der fuchsinschwefligen Säure leicht zu röten. Insbesondere die Chromocentren der am stärksten gelappten Kerne pflegen unverästelt zu sein (Abb. 1g und h; Abb. 2b, links oben; Abb. 3a).

Am seltensten sind Kerne, bei denen außer den Chromocentren auch die euchromatischen Abschnitte teilweise oder sogar gänzlich gefärbt sind. Ich habe nur einige solche Kerne gefunden. Gleich dünnen,

gewundenen Fäden bilden die Euchromatinteile eine verwickelte Strähne, in der man jedoch den Lauf mancher einzelnen Chromosomenarme weithin verfolgen kann (Abb. 1f; Abb. 3d). Durch derartige Chromatinfiguren kann man wenigstens bei den *Aphodius*-Arten schließen, daß die Chromocentren durch Verschmelzen der heteropyknotischen Proximalabschnitte verschiedener Chromosomen entstanden sind. Demgemäß

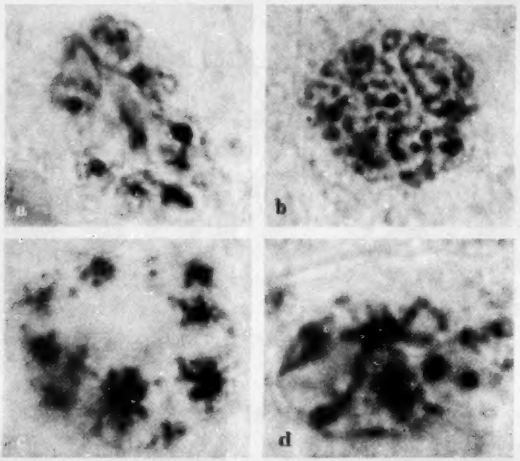

Abb. 3a—d. Chromocentren in verschiedenen Stufen ihrer Nukleinisierung bei *Aphodius foetens* (a, c, d) und *A. fimetarius* (b). a Unverästelte Chromocentren. Derselbe Kern wie in Abb. 1g. b Chromocentren mit halbnukleinisierten Ästen. Derselbe Kern wie in Abb. 1a. c Verästelte Chromocentren, von denen dieser Kern 12 enthielt. d Ein Chromocentrum in vollnukleinisiertem Zustand. a und c 2150mal, b und d 2866mal. San Felice; Feulgen + Lichtgrün.

sind sie also den Chromocentren des Diplotäns grundsätzlich gleich. Ein und dasselbe Chromosom besitzt kaum mehr als einen umfangreicheren heteropyknotischen Abschnitt. Dagegen kann es wohl kleinere allocyclische Abschnitte geben, die an der Chromocentrenbildung teilnehmen können. Die interchromosomalen Verbindungen können somit ziemlich verwickelt sein. In einigen großen hämatoxylingefärbten Kernen habe ich ein wirkliches Chromatinnetz gefunden; diese Färbung erlaubt aber nicht eine zuverlässige Deutung solcher Figuren (Abb. 1e).

In allen Interstitialzellen sieht man bei geeigneter Färbung Nukleolen. Ihre Zahl und Größe variieren nur in engen Grenzen. Verbindungen mit den Chromocentren sind nur selten feststellbar gewesen (Abb. 1e und 2b, c). Die Interstitialzellen zweier Arten, *A. rufipes* und *A. haemorrhoidalis*, sind besonders reich an Nukleolen. Dieselben Arten bilden

auch während der diplotänen Wachstumsphase der Spermatocyten ungewöhnlich große Nukleolen.

Trotz ihrer beträchtlichen allgemeinen Formvariabilität sind die Chromocentren in ein und demselben Kern praktisch gesehen immer gleich. Dagegen können Kerne von gleicher Größe verschiedenförmige und wiederum Kerne von verschiedener Größe auch sehr gleichförmige Chromocentren enthalten. Bei *A. fimetarius* schien es mir, als variiere die Häufigkeit verschiedener Chromocentrentypen von einem Individuum zum anderen. Betreffs der sternförmigen Chromocentren, die stets häufig vorhanden sind, ist dies schwieriger zu beobachten; aber das ungewöhnlich häufige Vorkommen z. B. solcher Chromozentren wie in Abb. 3b ist auffallend. Weil alle Individuen von sehr gleichmäßigem Alter sind, ist es schwer vorstellbar, daß zwischen den verschiedenen Individuen einerseits und zwischen den verschiedenen Chromocentrentypen andererseits irgendwelche grundlegenden Unterschiede bestehen. Wahrscheinlich haben wir es nur mit einem Phasenunterschied und einem leichten Synchronismus der Phasen zu tun. Ein dem mitotischen Cyclus ähnlicher Nukleinisations-Denukleinisationscyclus scheint wirklich das Chromatin der Interstitialzellen zu beherrschen. Weil ich kein einziges Anzeichen von eigentlichen Mitosen beobachtet habe, haben wir es hier zweifelsohne mit einem endomitotischen Cyclus zu tun. — Vor dem Deutungsversuch dieser Phasen sowie der Kernstruktur müssen einige ergänzende quantitative Feststellungen angeführt werden.

Die Chromocentren der Interstitialzellen sind im allgemeinen so deutlich und befinden sich so weit voneinander entfernt, daß die Bestimmung ihrer Zahl kaum weitere Schwierigkeiten als die durch die unvermeidliche Änderung der Fokuseinstellung verursachten darbietet. Bei den plattesten Kernen gelingt die Bestimmung im besten Falle fast mit einer einzigen Einstellung. Ausgezählt wurden nur Kerne mit unverästelten oder deutlichen, soliden, sternförmigen Chromocentren, weil die Zahl bei solchen Kernen wie dem in Abb. 3b oft unsicher bleibt. In bezug auf die Größe hat keinerlei Auswahl stattgefunden. Die Zählungen umfassen 8 Arten, bei denen der Kernbau am klarsten gewesen ist. Bei jeder Art wurden 100 Kerne, durchschnittlich 20 je Individuum, untersucht. Dabei fanden die Feulgen-Präparate natürlich stets den Vorzug. Bei *A. merdarius* und *A. haemorrhoidalis* war ich jedoch, um alle 100 Zellen zu finden, gezwungen, auch Kristallviolett- oder sogar Eisenhämatoxylinpräparate mitzunehmen. Dadurch sind kaum beträchtlichere Fehler entstanden, weil sich auch bei der Hämatoxylinfärbung der Nukleolus wegen seiner glatteren Oberfläche oder seiner Größe von den Chromocentren unterscheiden läßt.

Die Ergebnisse sind aus Abb. 4 ersichtlich[1]. Eine Mehrgipfeligkeit der Kurven, wie sie bei endomitotischer Polyploidisierung unter Ver-

[1] Schon früher (1949) habe ich bei 6 Arten die Chromocentrenzahl gezählt und dabei dieselben Präparate wie jetzt benutzt. Bei der damaligen Zählung erfolgte eine weniger strenge Auswahl betreffs der Deutlichkeit der Kernstruktur. Die Treppenkurven der Abb. 4 stellen die Ergebnisse jener Zählung dar. Ohne jeden Zweifel ist eine beträchtliche Menge von Zellen im Material von damals und heute gemeinsam.

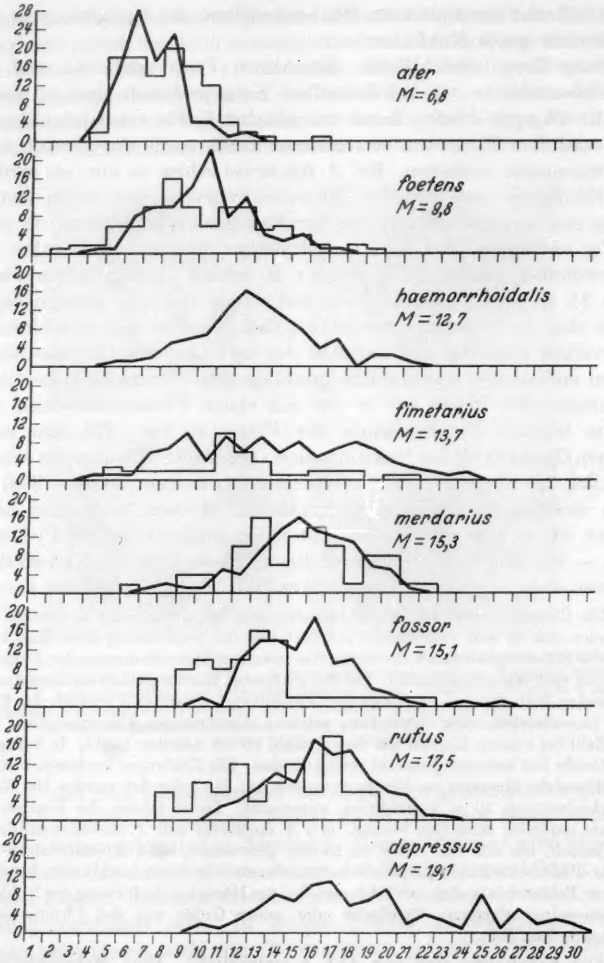

Abb. 4. Variation der Chromocentrenzahl in den Kernen der Interstitialzellen verschiedener *Aphodius*-Arten. Waagerecht: Chromocentrenzahlen; senkrecht: Kernzahlen. Mittelwerte unter den Artnamen. Die ältere Zählung ist durch eine Treppenkurve wiedergegeben.

vielfachung der Chromocentrenzahl erwartet werden könnte, liegt nicht vor. Zwar ist die Streuung auch bei diesen Kurven meistens viel größer als bei der idealen Zufallskurve; von einem Einfluß vervielfachter Zahlen-

klassen kann aber nicht die Rede sein. In der Tat ist die Streuung auffallend klein geblieben, wenn man bedenkt, daß die betreffenden Kerne in bezug auf ihre Größe zweifelsohne in zahlreiche Klassen fallen. Sie ist so geringfügig, daß die Kurven, zumal da eine unbestrittene Gipfelbildungstendenz existiert, die Variation einer einzigen Chromocentrenzahl bei Anwesenheit verhältnismäßig starker Dispersionsfaktoren bezeichnen können. Obschon die die Volumenverhältnisse nicht berücksichtigende Abb. 4 unzulänglich für weitere Erörterungen ist, können wir schon auf Grund derselben 3 wichtige Feststellungen machen:

1. Die Chromocentrenzahl der Interstitialzellen wird bei Vervielfachung des Kernvolumens sehr wahrscheinlich nicht vervielfacht. 2. Die Zahl bleibt im allgemeinen kleiner als die diploide Chromosomenzahl 20. 3. Es gibt Artunterschiede bezüglich der Zahlenvariation.

B. Das Verhältnis der Chromocentrenzahl zum Kernvolumen.

Es war nun notwendig, trotz der Verschiedenförmigkeit der Kerne wenigstens ein grobes Bild von den Volumenverhältnissen zu gewinnen, um festzustellen, ob die Chromocentrenzahl überhaupt vom Kernvolumen abhängig ist oder nicht. Zu diesem Zwecke habe ich die klarkernigen Arten *A. foetens* und *A. fimetarius* untersucht, bei denen die Variation der Chromocentrenzahl zugleich ziemlich verschieden ist. Bei den ersteren habe ich die Chromocentrenzahl und in groben Zügen auch das Kernvolumen von 152 Zellen bestimmt, bei der letzteren nur von 57 Zellen, die jedoch nach möglichst gleichmäßiger Kernform ausgewählt wurden.

Betreffs der Deutlichkeit der Chromocentren wurden an die Kerne dieselben Forderungen wie oben gestellt, aber in bezug auf die Form ist es notwendig gewesen, schon bei *A. foetens* eine ziemlich strenge Selektion durchzuführen. Alle Kerne, die im Präparat von oben gesehen sichtlich schräg lagen, sind außer acht gelassen worden, ebenso die beträchtlich kegelförmigen und alle gebogenen, verästelten und sehr buckeligen Kerne. Dadurch sind etwa 50% der Kerne ausgemustert worden. Alle Kerngrößenklassen sind dessenungeachtet ohne jeden Zweifel im Material repräsentiert, die größeren Klassen allerdings wahrscheinlich weniger zahlreich, als es ihrer Häufigkeit entspricht. Die Formunregelmäßigkeit scheint nämlich bei den größeren Kernen häufiger als bei den kleineren zu sein.

Von den gewählten Kernen wurden die Umrisse (der maximale Flächeninhalt) unter Anwendung des Abbe-Apparates auf der Höhe des Arbeitstisches auf das Papier übertragen. Die Vergrößerung wurde mit dem Objektmikrometer bestimmt und ist eine 2805fache. Die „Tiefe" des Kernes wurde mittels der Mikrometerschraube bestimmt. Gemäß der Skala der Mikrometerschraube beträgt die Größe der Tiefeneinheit 2 μ. Diese Skala wurde durch Vergleichen der Dicke eines Haares bei gleicher Vergrößerung mit dem Abbe-Apparat auf das Papier übertragen und mittels der

Mikrometerschraube als Tiefe messend nachgeprüft und als zuverlässig gefunden. Die Bestimmung des obersten und untersten Punktes des Kernes ist durch die periphere Lage der Chromocentren sehr erleichtert worden.

Die gezeichneten Flächeninhalte der Kerne wurden dann mit dem Planimeter in zweimaligem Umgang gemessen. Die Flächeninhaltzahlen werden dadurch verdoppelt. Wie unten hervorgehen wird, lassen sich diese Zahlen als solche gebrauchen. Weil hier eigentlich keine größere Genauigkeit als die zur Ermittlung verschiedener Polyploidieklassen notwendige erstrebt wurde, und weil die große Formvariation auch bei den gewählten Kernen die Anwendung einer genaueren, einheitlichen Methode auf jeden Fall unmöglich macht, habe ich mich mit einer ganz groben Methode begnügt. Der Flächeninhalt ist nur mit der Tiefe multipliziert worden. Das Volumen ist in μ^3 angegeben und die Rechnung in der Praxis nach der folgenden Formel ausgeführt worden (V Volumen; A der mit dem Planimeter bestimmte doppelte Flächeninhalt, T Tiefe in Einheiten der Mikrometerschraube):

$$V = 2T\left[\frac{\sqrt{\frac{A}{2}}\cdot 10000}{2805}\right]^2 = 2T\left(\sqrt{\frac{A}{2}}\right)^2\cdot\left(\frac{10000}{2805}\right)^2 = TA\cdot 12{,}68.$$

Das tatsächliche Volumen ellipsoidischer und kugeliger Kerne beträgt $^2/_3$ vom so errechneten Wert; bei den mehr abgeplatteten Kernen bleibt der Unterschied kleiner.

Bei *A. fimetarius* wurden nur beinahe regelmäßige kugelige oder rotationsellipsoidische Kerne ausgewählt. Hier ist die Volumenbestimmung folglich mit weit größerer Zuverlässigkeit durchführbar. Um die Vergleichbarkeit zu erhalten, ist die von der Ellipsoidformel vorausgesetzte $^2/_3$malige Verkleinerung jedoch auch hier weggelassen worden. In beiden Materialien dürften sich die Volumenklassen nicht so sehr vermischen, daß die eventuelle Mehrgipfeligkeit der die Volumenvariation angebenden Kurve verwischt würde.

Bei *A. foetens* beträgt die Variationsbreite 2000 μ^3, während die kleinsten Kerne 20—60 μ^3 groß sind. Der weit überwiegende Teil der Variation fügt sich zwischen 20 und 900 μ^3 ein, aber auch diese Strecke ist weder eingipflig noch gleichmäßig frequentiert. Möglicherweise enthält sie 3 oder 4 Gipfel (Abb. 5, *I*).

Wird eine in bezug auf die kleinsten Kernvolumina zu große Klassenbreite angewandt, so werden die ersten verdoppelten Volumenklassen natürlich leicht maskiert. Die Mehrgipfligkeit des vorderen Teiles wird auch bei dieser Kurve erst bei Anwendung einer halb so großen Klassenbreite erkennbar (Abb. 5, *II*). Der 1. Abschnitt der Kurve besteht aus 8 Kernen, deren durchschnittliches Volumen 45 μ^3 beträgt. Die Vielfachen dieser Zahl sind 90, 180, 360, 720 usw. Nun bemerkt man, daß die Gipfel der Kurven faktisch nahe bei den genannten Zahlen liegen; eine beträchtlichere Abweichung wird nur durch die große Frequenz der Klasse 560—600 verursacht. Ohne jeden Zweifel haben wir es hier mit einer Wirkung verschiedener Polyploidieklassen zu tun, obschon die Grobheit der Methode einerseits und die Wirkung der noch wachsenden Kerne andererseits die Mehrgipfligkeit in der Kurve nicht einwandfrei hervortreten lassen (vgl. Voss 1948). Sind die kleinsten Zellen diploid, wie dies wohl anzunehmen ist, so sind die größten 64ploide oder solche

128ploide, die ihre endgültige Größe noch nicht erreicht haben. Es ist natürlich möglich, daß die kleinsten Zellen nur haploid sind. Wichtig ist, daß wir verschiedene Polyploidiestufen festgestellt und soweit von-

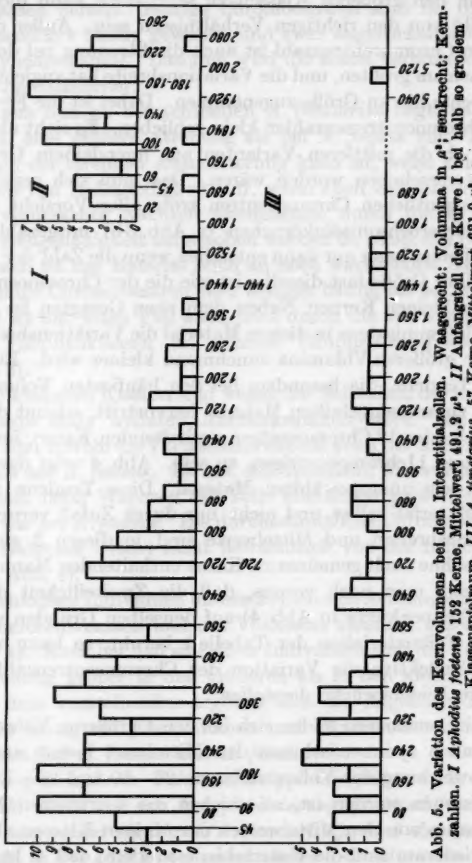

Abb. 5. Variation des Kernvolumens bei den Interstitialzellen. Waagerecht: Volumina in μ^3; senkrecht: Kernzahlen. *I Aphodius foetens*, Mittelwert 491,3 μ^3. *II* Anfangsteil der Kurve I bei halb so großem Klassenspielraum. *III A. fimetarius*, 57 Kerne, Mittelwert 621,0 μ^3.

einander unterschieden haben, daß wir die Chromocentrenzahlen bei denselben vergleichen können.

In der Tabelle 1 (S. 14) ist das Material in 6 aufeinanderfolgende Portionen geteilt worden, so daß jede von diesen eine vermutete Polyploidieklasse enthält. Bei einem Blick auf diese Tabelle ergibt sich sogleich die

Richtigkeit unserer oben gemachten Annahme, daß keine Vervielfachung der Chromocentrenzahl mit der Polyploidisierung verbunden ist. Im Gegenteil scheint die Zahl in den kleineren Klassen durchschnittlich sogar größer als in den größeren Klassen zu sein; doch kann dies ein verfälschtes Bild von den richtigen Verhältnissen sein. Außer der durchschnittlichen Chromocentrenzahl ist auch die Streuung bei den niedrigsten 2 Klassen am größten, und die Variationsbreite hat zugleich namentlich nach rechts hin an Größe zugenommen. Dabei ist die Frequenz der mittleren Chromocentrenanzahlen klein geblieben. Es sieht also beinahe so aus, als ob die mittleren Varianten aus irgendeinem Grunde weit nach rechts verschoben worden wären. Das muß sich ergeben, wenn außer den eigentlichen Chromocentren trotz aller Vorsicht auch subproximale Heterochromatinkörnchen (s. Abb. 3b) mitgezählt werden. Ein solcher Fehler kann nur dann entstehen, wenn die Zahl der Körnchen gering und ihre Größe fast dieselbe ist wie die der Chromocentren, d. h. besonders in kleinen Kernen. Neben dem eben Gesagten ist jedoch zu bemerken, daß wenigstens in diesem Material die Variationsbreite bei den Klassen der größeren Volumina zunehmend kleiner wird. Eine weitere allgemeine Tendenz, die besonders bei den häufigsten Volumenklassen sowie beim zusammengefaßten Material hervortritt, scheint die Häufigkeit der 6—8 oder 12 Chromocentren enthaltenden Kerne, im Vergleich mit den 9- und 11chromocentrigen, zu sein. Abb. 4 zeigt dasselbe Phänomen aus dem unausgewählten Material. Diese Tendenz muß wohl durch das Material selbst und nicht nur durch Zufall verursacht sein. Die Variationsbreiten und Mittelwerte sind in diesen 3 gleichartigen und zweifelsohne auch gemeinsame Kerne enthaltenden Materialien sehr ähnlich. Setzt man noch voraus, daß die Zweiteiligkeit des älteren Materials (Treppenkurve in Abb. 4) auf denselben Gründen wie die der 2 kleinsten Teilmaterialien der Tabelle 1 beruht, so kann man sagen, daß die 3 Kollektive die Variation der Chromocentrenzahl ohne beträchtlichere Widersprüche darstellen[1].

Die Variationskurven bilden sich bei den 4 größeren Volumenklassen jedoch ziemlich symmetrisch um den Mittelwert herum aus. Erklärt sich die Abweichung der Volumenklassen 20—80 und 80—130 wirklich wie angenommen worden ist, so weichen die korrigierten Mittelwerte derselben kaum von den Mittelwerten der übrigen Klassen ab. Bei den letzteren wiederum sind die Unterschiede so klein, daß es kein Wunder wäre, wenn sie mit der Vermehrung des Materials verwischt würden.

[1] Es sei noch erwähnt, daß ich schon früher aus denselben *A. foetens*-Präparaten 50 zu den kleinsten und ebenso viele zu den größten gehörende Kerne ausgewählt und bei ihnen die Chromocentren gezählt habe. Bei den kleinen Kernen war die Variationsbreite 6—17 und der Mittelwert 10,2, bei den großen entsprechend 5—18 und 10,0.

Tabelle 2 zeigt die Variation der Chromocentrenzahl bei verschiedenen Individuen. Das Individuum Nr. 4 stellt sich als der eigentliche Urheber der besprochenen Unregelmäßigkeiten bei den kleinsten Volumenklassen heraus. Fetzige Chromocentren sind bei diesem Individuum besonders häufig gewesen, und zwar augenscheinlich wegen eines Phasensynchronismus. Dadurch wird die schon vermutete Fehlerquelle noch wahrscheinlicher.

Bei dem nun zu betrachtenden *A. fimetarius* liegt, nach Abb. 4 zu schließen, eine größere Streuung als bei *A. foetens* vor. Das Material, das wegen der strengen Ausmusterung klein ist, weist keine deutlichen Anzeichen von Mehrgipfligkeit auf, wenn auch der durch die Materialauslese verursachte Nachteil zweifelsohne durch die in diesem Falle größere Meßgenauigkeit aufgehoben worden ist (Abb. 5, *III*). Allem Anschein nach ist das Material reich an noch wachsenden Kernen. Solide, sternförmige Chromocentren sind weniger häufig als bei *A. foetens*. Das Material ist auf jeden Fall auch jetzt durch die am wenigsten frequentierten Volumenklassen in mehrere Gruppen aufgespalten worden (Tabelle 3).

Die 2 kleinsten Klassen sind wegen der Seltenheit der entsprechenden Fälle kaum einer weiteren Aufmerksamkeit wert. Bei den übrigen Klassen sind sowohl die Variationsbreite als auch der Mittelwert deutlich größer als bei *A. foetens*. Es hat den Anschein, als würde die Chromocentrenzahl beim Wachsen der Zelle allmählich größer, also ganz umgekehrt wie bei *A. foetens*. Die durchschnittliche Chromocentrenzahl des ganzen Materials weicht nicht beträchtlich von der früher bestimmten ab (vgl. Abb. 4).

Verschiedene Individuen unterscheiden sich in bezug auf die Variation der Chromocentrenzahl und des Volumens ziemlich stark voneinander (Tabelle 4). Auch bei den einzelnen Individuen sind die Variationsbreite und Streuung größer in den größeren als in den kleineren Kernen.

Aus dem vorstehenden ergeben sich die folgenden Hauptfeststellungen in bezug auf Kernvolumen und Chromocentrenzahl der Interstitialzellen bei *A. foetens* und *A. fimetarius*:

1. Es gibt mehrere Volumen(Polyploidie-)klassen. 2. Die Chromocentrenzahl bleibt bei der Polyploidisierung praktisch unverändert (A. foetens) oder nimmt etwas zu (A. fimetarius), wird aber auf keinen Fall verdoppelt. 3. Die Zahl ist bei verschiedenen Individuen von gleichem Alter praktisch dieselbe (A. foetens) oder schwankt etwas (A. fimetarius).

Das Obengesagte gilt zweifelsohne auch für die anderen untersuchten Arten. Es möge noch auf 3 wichtige, das ganze Material betreffende Tatsachen hingewiesen werden:

1. Die Polyploidisierung findet am wahrscheinlichsten durch Endomitose statt. 2. Die Kerne weisen keinerlei Anzeichen der Endoanaphase,

Tabelle 1.

Volumenklasse (μ^3)	Anzahl der Kerne	Anzahl der Chromocentren in den einzelnen Kernen																				Im Mittel
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
20— 80	8						1	1		2							1	1	1		1	12,8
80— 130	11									2	2			2		2		1	1		1	13,6
130— 320	35				2	2	1	7	5	1	7	4	1	2	3							9,8
320— 530	34			1		1	5	2	3	5	5	3	6	2			1					9,4
530—1080	51						3	6	4	5	15	2	8	2	4	2						10,1
1080—2020	13								3	2	1	4	2		1							10,3
	152			1	2	3	10	16	15	17	30	13	17	8	8	4	2	2	2		2	10,1

Tabelle 2.

Individuum Nr.	Anzahl der Kerne	Volumen (μ^3)		Anzahl der Chromocentren in den einzelnen Kernen																		Im Mittel
		Variationsbreite	im Mittel	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	36	57—1254	540,0				1		3	3	3	9	11	2	2			2						9,2
2	44	26—2011	343,1						3	4	3	4	9	5	8	4	2	1	1					10,3
3	40	101—1824	600,0			1	1	2	2	3	4	3	9	5	3	3	4							9,9
4	32	30— 828	325,4					1	2	6	5	1	1	1	4	1	2	1	1	2	2		2	11,2
	152	26—2011	408,0			1	2	3	10	16	15	17	30	13	17	8	8	4	2	2	2		2	10,1

Tabelle 3.

Volumenklasse (μ^3)	Anzahl der Kerne	Anzahl der Chromocentren in den einzelnen Kernen																										Im Mittel
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24–29	30	31	32	
30— 70	3									1		2																	10,3
70— 150	4									1		1		2															11,5
150— 340	18							1	2			4	3	2	1	1		1	2		1								12,8
340— 700	15				1	1		2	1		1		3	1			1		1		1	1				1			13,0
700—1560	16				1	1	1			2	2	1	1	1		1	1		1	1		1		1					12,6
5085	1																											1	(32,0)
	57				2	2	1	3	3	4	3	8	7	6	1	2	2	1	4	1	2	2		1		1		1	13,1

Tabelle 4.

Individuum Nr.	Anzahl der Kerne	Volumen (μ^3)		Anzahl der Chromocentren in den einzelnen Kernen																									Im Mittel
		Variationsbreite	im Mittel	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24–29	30	31	32	
1	17	55— 413	225,0									1	2	3	2	4		1		1	2		1							13,4
2	18	53—1305	736,4				1	2	1	1	1	3	1	2	3		1		1					1						10,1
3	7	55— 598	255,3							1	1			3	1				1											10,8
4	15	388—5085	1165,0				1			1	1				1	2		1			2	1	1	1		1		1		1	17,8
	57	53—5085	621,0				2	2	1	3	3	4	3	8	7	6	1	2	2	1	4	1	2	2		1		1		1	13,1

d. h. des Sich-Trennens endomitotisch entstandener Chromosomen auf. 3. Die Chromocentren sind in kleineren Kernen durchschnittlich kleiner als in größeren.

Diese Feststellungen zusammen mit den obenerwähnten leiten unvermeidlich zu einer Schlußfolgerung, die sich wohl schon auf Grund des Baues der Chromatinfiguren vermuten ließ: *Die Polyploidie der Interstitialzellen entwickelt sich durch successive Endomitosen, bei denen die entstandenen Tochterchromosomen wegen einer strengen partiellen*

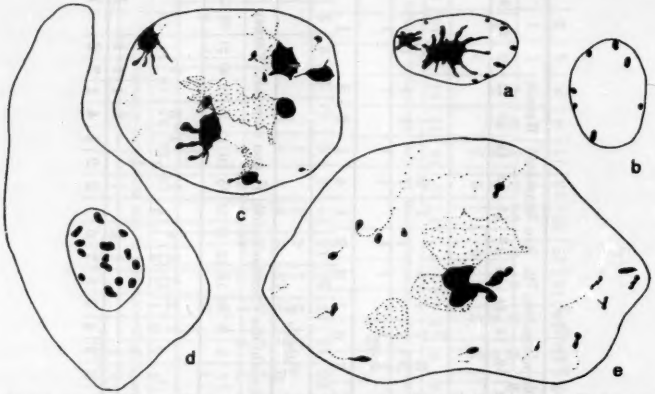

Abb. 6a—e. Zellen und Kerne aus verschiedenen somatischen Geweben des Hodens von *Aphodius fociens* (a), *A. rufipes* (b), *A. fimetarius* (c) und *A. haemorrhoidalis* (d, e). a und b Vas deferens-Kerne. c Kern aus der peritonealen Hodenhülle. d Junge Sekretzelle aus der Vas deferens-Kapsel. e Kern einer ausgewachsenen Sekretzelle. San Felice; Feulgen, in c und e dazu Lichtgrün.

Heteropyknose beisammenbleiben und somit allmählich wachsende Sammelchromocentren bilden.

2. Die anderen somatischen Zellen des Hodens.

Die neben den Interstitialzellen vorhandenen anderen Somazellen des Hodens eignen sich weniger gut für die Kernanalyse. Entweder sind die Zellen von gleicher Polyploidiestufe oder die Chromocentren unklare, massige Klumpen. Hier werden nur die Hauptzüge der Kernstruktur vergleichshalber dargestellt.

a) *Die Zellen der Außenmembran und die Cystenzellen.* Für beide Zellarten ist charakteristisch die Kleinheit der meistens körnchenförmigen Chromocentren, deren Zahl gewöhnlich unter 10 bleibt. Zusammentreten zu größeren Sammelchromocentren findet besonders bei den Cystenzellen, die sich augenscheinlich endomitotisch entwickeln, statt. Sie sind auch ziemlich reich an Nukleolarsubstanz. Die Membranzellen sind

abgeplattet und sicherlich alle von gleicher Polyploidiestufe, höchstwahrscheinlich diploid (Abb. 7b).

b) Die *Zellen des Vas deferens* sind vorwiegend von gleicher Größe; Kerne mit etwa doppeltem Volumen findet man jedoch im Ende des kapselartigen Anfangsteils nahe der Region der Spermatogonien. Die Kerne sind überhaupt sehr regelmäßig. Ich habe zum Vergleich bei *A. foetens* aus 10 Zellen von üblicher Größe das Kernvolumen mit der S. 9—10 angegebenen Methode bestimmt und den Durchschnittswert von 170 μ^3 erhalten. Dieser weicht nicht sehr von dem der 3. Volumenklasse

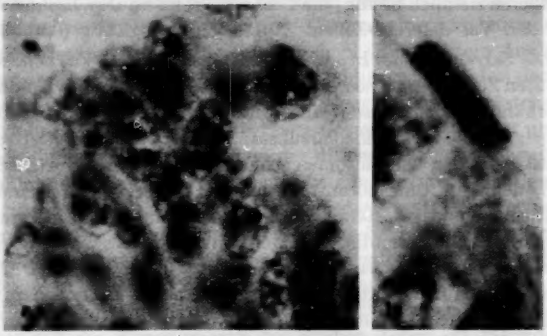

Abb. 7a u. b. Kernstruktur zweier Somazellarten bei *A. fimetarius*. a Verästelte Chromocentren in den Wandzellen des kapselartigen Anfangsteils des Vas deferens (1433mal; San Felice; Feulgen + Lichtgrün). b Unverästelte Chromocentren im Kern einer Hodenmembranzelle (2150mal; San Felice; Eisenhämatoxylin).

der Interstitialzellen (vgl. Abb. 5, *I*) ab. Die Vas deferens-Kerne wären somit im allgemeinen tetra- oder oktoploid, je nachdem ob die kleinsten Interstitialzellen haploid oder diploid sind. Polyploidie bis zur Oktoploidie ist auch früher bei den Vas deferens-Zellen festgestellt worden (z. B. GEITLER 1943).

Die Chromocentrenzahl variiert auch bei diesen Zellen innerhalb viel engerer Grenzen als bei den Interstitialzellen. Bei *A. fimetarius* fand ich in 50 Kernen durchschnittlich 1,8 Chromocentren, während sich die Variationsbreite auf 1—5 Chromocentren erstreckte. Bei *A. foetens* und *A. fossor* ergab doppelt so großes Material entsprechend die Werte 4,1 (1—7) und 7,3 (1—13). In den meisten Fällen sind die Chromocentren verästelt und im Vergleich zur Kerngröße stattlich (Abb. 6a und 7a). Allem Anschein nach befinden sich sämtliche proximalen Chromosomenabschnitte in einem nukleinisierten Zustand. Daraus folgt weiterhin, daß die Chromocentren keine Homologengruppen, sondern gemischte Aggregationen sind. Bisweilen und namentlich bei *A. rufipes* begegnet

man auch Kernen ohne Sammelchromocentren. Solche Kerne, die peripher an der Kernmembran befindliche kleine Chromocentren enthalten (Abb. 6b), weisen darauf hin, daß es art- und zustandsbedingte Verschiedenheiten auch in der Kernstruktur der Vas deferens-Zellen gibt.

c) *Die Zellen der peritonealen Hodenhülle.* Die Hülle ist nicht immer mitpräpariert worden. Die Zellen und ihre Kerne waren in allen Teilen der Hülle von gleicher Größe. Ihr Volumen scheint etwa achtmal so groß zu sein wie das der Vas deferens-Zellen. Die Chromocentren sind mehr oder minder deutlich verästelt. Kleine Chromocentren sind neben den größeren ziemlich häufig; die Gesamtzahl ist im Durchschnitt größer als bei den Vas deferens-Zellen. Nukleolen sind häufig (Abb. 6c).

d) *Die Sekretzellen des kapselartigen Anfangsteils des Vas deferens* sind die größten Zellen des *Aphodius*-Hodens. Verschiedene Polyploidiestufen sind vorhanden, doch herrscht eine bestimmte Größe vor. Der Kerninhalt ist wegen reichlicher Nukleolarsubstanz oft schwer analysierbar. Die Despiralisation der Chromosomen scheint besonders weit fortgeschritten zu sein, denn die Chromocentren sind nur selten verästelt und dazu verhältnismäßig klein. Bisweilen findet man im Kern mehrere kleine Heterochromatinkörper, die eine Heteropyknose der distalen Chromosomenenden vermuten lassen (Abb. 6e). Zusammentreten der Chromocentren ist ziemlich häufig. Unter den kleinsten Sekretzellen gibt es auch solche, deren Chromosomenverhältnisse mehr denen der Interstitialzellen ähneln (Abb. 6d). Solche Zellen haben sicherlich noch mehrere Endomitosen vor sich.

Außer in den somatischen Hodenzellen, habe ich auch in den ausnahmsweise in die Präparate geratenen Zellen des Darmes und der MALPIGHIschen Gefäße verästelte Chromocentren gesehen.

IV. Diskussion.

1. Die Aphodius-Arten als Chromocentrenbildner.

A. Vergleich zwischen Spermatocyten und Somazellen.

Bei der Untersuchung der Spermatogenese von 15 Arten der Gattung *Aphodius* (VIRKKI 1951) habe ich gefunden, daß die Heteropyknose der Centromerenregionen eine vom Ende des Pachytäns bis zur Diakinese andauernde Assoziation der proximalen Bivalentenabschnitte verursacht. Auf der Entstehungsweise solcher Chromocentren beruht es, daß die Form der Gebilde einem Stern ähnlich ist: ein kompaktes Zentrum mit strahlenartigen Ausläufern. Während des diffusen Diplotäns bleibt aber nur das Zentrum deutlich sichtbar, und zwar weil im Euchromatin eine mehr oder minder vollständige Despiralisation stattfindet. Der diploide Chromosomensatz besteht aus 20 metacentrischen Chromosomen, die hauptsächlich nur durch ihre Größe voneinander ab-

weichen. Auch die Geschlechtschromosomen sind mit 2 euchromatischen Armen versehen. Die proximale Heteropyknose bildet sich aus zusammen mit dem Bukett und wird in der diakinetischen Renukleinisationsphase nicht mehr wahrnehmbar. Negative Heteropyknose ist nicht beobachtet worden.

Die Chromocentren der Interstitialzellen sind in ihrer Form denen der Spermatocyten so ähnlich, daß man einen prinzipiell gleichen Bau bei beiden vermuten muß. Weil in der Spermatogenese die Centromerenregionen aller Chromosomen — und nur sie — heteropyknotisch waren und dabei Assoziationsneigungen erkennen ließen, kann man kaum bei den Somazellen andere als proximale Chromosomenabschnitte als Urheber der Chromocentrenbildung anzusehen. Eine weitere Analogie ist im Phasenwechsel der Chromocentren zu erblicken: sowohl in den Interstitialzellen als in den Spermatocyten begegnet man verästelten *und* unverästelten Chromocentren. Die Phasenfolge der Meiose zeigt unmittelbar, daß die unverästelten Formen der diffusen Wachstumsphase eigen sind, während die verästelten Formen nur in jüngeren, noch gut färbbaren Spermatocyten vorkommen. Man darf wohl den Formwechsel der Chromocentren bei beiden Zellkategorien für gleichartig halten. Die Interstitialzellen mit unverästelten Chromocentren befänden sich somit in voller interphasischer Tätigkeit. Ohne jeden Zweifel haben wir in diesem Falle eine *Endointerphase* vor uns. Die früher erwähnte Beobachtung, daß die Kernmembran bei solchen Zellen oft besonders unregelmäßig ist, kann ebenso ein Ausdruck von reger Tätigkeit sein, vergrößert ja die Gelapptheit die wirksame Oberfläche des Kerns. Wie bekannt, weisen physiologisch aktive Zellen öfters lappige oder verästelte Kerne auf. Die Zellen mit sternförmigen Chromocentren wiederum werden am besten als *prophasisch* gedeutet. Diese Auffassung wird auch durch die Beobachtungen von Lauber (1947) an polyploiden Kernen der Pflanze *Blumenbachia* gestützt. Beim Eintritt der Prophase der künstlich erzeugten Mitose wandeln sich nämlich die unverästelten, interphasischen Chromocentren in verästelte, prophasische um.

Weil der größte Teil der Interstitialzellen auf der Endoprophase zu verharren scheint, muß dieses Stadium von langer Dauer sein. Jedoch haben nur die subproximalen Abschnitte die Neigung, in einem halbnukleinisierten Zustand aufzutreten, während die distaleren Teile fast immer interphasisch, also völlig despiralisiert bleiben. Jene ziemlich seltenen Fälle, in denen alles Euchromatin sichtbar zu sein scheint, stellen wohl der Endometaphase nahe Phasen dar. Darauf weist auch die Seltenheit solcher Kerne hin. Bei ihnen ist die stärkere Färbbarkeit der subproximalen Chromosomenabschnitte nicht mehr feststellbar. Es scheint also, daß die letzteren nur in der lange dauernden Prophase allocyclisch sind.

Aus dem Vergleich eines *Aphodius*-Chromosoms in verschiedenen Nukleinisationsphasen der Soma- und Spermatocytenkerne ergibt sich, daß wir jeden Arm in 4 ungleiche Abschnitte einteilen, und zwar 1. einen kurzen Abschnitt dicht beim Centromer, 2. einen etwa 3—4mal längeren Abschnitt distal davon, 3. einen Zwischenabschnitt, der den weitgrößten Teil des Armes umfaßt, 4. einen ganz kurzen Distalabschnitt. Der 1. Abschnitt ist in allen Kernphasen nukleinisiert. Er scheint mit dem entsprechenden Abschnitt des anderen Armes zu verschmelzen. Der 2. Abschnitt ist in den Spermatocyten kaum wahrnehmbar, wohl aber in den Interstitial-, Hodenhüllen- und Vas deferens-Zellen. Er bildet dort die Äste der Chromocentren. Er ist nicht immer an jedem Punkt von gleicher Dicke, sondern oft perlschnurartig, und die den Chromomeren ähnlichen „Perlen" können, wie wir gesehen haben, in gewissen Phasen wegen der Despiralisierung der Zwischenteile voneinander abgesetzt sein. Der 3. Abschnitt besteht aus dem eigentlichen Euchromatin und tritt bei allen Interphasen und interphasenartigen Zuständen des Kerns despiralisiert und denukleinisiert auf. Der Distalabschnitt endlich scheint eine schwache Neigung zur Heteropyknose in einigen Somazellen (bei *A. rufus* auch in der Spermatogenese) aufzuweisen.

B. Über eine vermutete Entstehungsweise der charakteristischen Kernstruktur der Interstitialzellen.

GRAFL (1939, 1940) und GEITLER (1939, 1940, 1941) haben an verschiedenen pflanzlichen und tierischen Objekten festgestellt, daß die durch die Endomitose entstandenen Tochterchromosomen leicht zusammenhängen bleiben, wenn sie heterochromatisch sind. Die Beobachtungen von PAINTER und REINDORP (1939) an *Drosophila melanogaster* weisen zudem darauf hin, daß die Heteropyknose dabei nicht besonders ausgeprägt zu sein braucht. Vorausgesetzt, daß der Kerninhalt durch keine Strömungen durchgemischt und die Tochterchromosomen durch keine beträchtlichere Repulsion auseinander getrieben werden, ist es natürlich möglich, daß auch völlig euchromatische Chromosomen nach den Endomitosen gleicherweise gruppiert bleiben. Bei den *Aphodius*-Arten ist es nicht überraschend, daß die Homologengruppen bei ihnen als selbständige Chromocentren bestehenbleiben; die Verklebungstendenz der heteropyknotischen Abschnitte allein ist ohne jeden Zweifel hinreichend, um die Sammelchromocentren in diesem Falle entstehen zu lassen.

Die Tatsache, daß die Zahl der Chromocentren die diploide Chromosomenzahl praktisch nicht überschreitet, muß meines Erachtens so gedeutet werden, daß sich die Zellen nach dem Beginn der endomitotischen Polyploidisierung nicht mehr mitotisch geteilt haben. Wäre nämlich

eine polyploide Mitose dazwischen eingeschaltet gewesen, so hätten die Homologen nach derselben eine völlige oder nahezu völlige Wiedervereinigung machen müssen; denn im entgegengesetzten Falle würde die Chromocentrenzahl die 20er Grenze weitaus überschreiten. Eine so vollständige Wiedervereinigung ist aber ziemlich schwer vorstellbar, weil die Summe der Homologen wegen der Polyploidisierung gestiegen ist. Und kaum leichter vorstellbar wäre eine fortdauernd so kleine Chromocentrenzahl, wenn die Wiedervereinigung unabhängig von der Homologie der Chromosomen geschehe. Es scheint somit am wahrscheinlichsten, daß die Chromocentren von der diploiden Stufe des Kerns herstammen. Die Interstitialzellen gehören ohne jeden Zweifel zu denjenigen Polyploidzellen, die ihr mitotisches Teilungsvermögen verloren haben (s. OKSALA 1939).

Vergegenwärtigen wir uns die letzte diploide Interphase vor der ersten Endomitose, so bestehen die folgenden 6 Möglichkeiten in bezug auf die Vereinigung der Proximalabschnitte: 1. Alle 20 Chromosomen sind frei, 2. manche Chromosomen sind zufallsbedingt vereinigt, 3. alle Chromosomen sind zu einem oder zu mehreren Centren zufallsbedingt assoziiert, 4. alle Homologen sind (völlig oder nur im Bereich der Proximalabschnitte) gepaart, 5. nur ein Teil von den Homologen befindet sich in gepaartem Zustand, 6. es finden sich sowohl durch die Homologie und den Zufall bedingte Aggregate als auch einzelne Chromosomen im Kern. — Welche von diesen Möglichkeiten bei den verschiedenen Arten tatsächlich verwirklicht ist, ist nicht sicher zu entscheiden. Einiges kann indessen aus der statistischen Analyse schon geschlossen werden.

Beim Betrachten der Zahlenvariation in Abb. 4 wird man von der Verschiedenheit der einzelnen Arten auch in dieser Hinsicht überzeugt. Wegen der durchgehend geringen Frequenz nahe bei der 20-Zahl ist es klar, daß sich die Chromocentren nur selten von den unvereinigten Chromosomen ableiten können. Nur unter der Voraussetzung, daß die so entstandenen Chromocentren später häufig zusammentreten, wäre ein solcher Anfangszustand verständlich. In den kleineren Zellen sind indessen keine wesentlichen Zahlenveränderungen feststellbar gewesen.

Die Möglichkeiten 4 und 5 sind besonders interessant. Entstehen die Chromocentren wirklich aus „somatischen Bivalenten" und tritt später keine Aggregation der Chromocentren ein, so entspricht die Zahl der letzteren in allen Polyploidieklassen der Zahl 10. Wir haben schon gesehen, daß mehrere Zählungen bei *A. foetens* den Mittelwert von etwa 10 ergeben haben. Dazu sind die Variationsreihen so gestaltet gewesen, daß man von einer zufallsbedingten Variation der 10-Zahl reden kann. Bei den meisten Arten liegen aber der Mittelwert und die am reichlichsten frequentierten Zahlenklassen etwas über der 10-Zahl. Dies wäre wohl durch die 5. Möglichkeit erklärbar.

Die Kerne mit sehr wenigen und großen Chromocentren zeugen andererseits dafür, daß auch die Nichthomologen oder aus verschiedenen Chromosomen stammenden Chromocentren zusammentreten können. So verhält es sich ja auch in der Spermatogenese. Es ist weiter klar, daß die ganze Variation unterhalb der 10-Zahl nur unter Berücksichtigung dieser Tatsache verständlich wird. Die die 20-Zahl überschreitende Variation ihrerseits ist ein Zeichen dafür, daß die Chromocentren und namentlich auch die Homologengruppen trotz ihrer „Klebrigkeit" zum Zerfallen befähigt sind.

Der Aufbau der Chromocentren scheint also ziemlich verwickelt zu sein. Am besten läßt sich von den obigen Möglichkeiten wohl die 6. zur Erklärung der Verhältnisse wählen. Dabei sind wir meines Erachtens genötigt, der Paarung homologer Chromosomen oder wenigstens ihrer Proximalabschnitte während der diploiden Stufe eine große Bedeutung als Grundereignis der Chromocentrenbildung beizumessen. Die Wirkung dieser Erscheinung bleibt trotz einer mehr oder minder großen Variation der Zahl erkennbar.

Die Chromocentrenzahl der Interstitialzellen ist jedoch keineswegs das einzige Zeichen von somatischer Paarung bei den *Aphodius*-Arten. Dahin weisende Beobachtungen habe ich nämlich schon früher an den ersten Spermatogoniengenerationen bei *A. fimetarius* gemacht (VIRKKI 1951, S. 31, 32 nebst Abb. 146 und 147). In den spätanaphasischen (eventuell frühprophasischen) Chromosomengruppen jener Gonien finden sich nicht 20, sondern 10 Elemente, wie wenn alle Homologen vollständig parallel konjugiert wären. Leider war mein aus Imagines gesammeltes Material schon zu alt für eine genauere Untersuchung dieses Phänomens. Die Nebeneinanderlagerung homologer Chromosomen in den Metaphaseplatten, die oft für ein hinreichendes Zeichen von der somatischen Konjugation angesehen wird, ist in den Gonienmetaphasen nie vollständig gewesen. Desto häufiger begegnet man aber bei allen *Aphodius*-Arten Spermatogonienmetaphasen, bei denen die Platte ein bis mehrere Paare sich nebeneinander befindender Homologen enthält. Die Unvollständigkeit der Nebeneinanderlagerung kann natürlich keineswegs als Beweis einer unvollkommenen somatischen Paarung gedeutet werden. SVÄRDSON (1941), nach dem die somatische Konjugation bei einigen von ihm untersuchten Fischen kein seltenes Phänomen ist, hat festgestellt, daß eine ausgeprägte Nebeneinanderlagerung in der Metaphaseplatte keine obligatorische Begleiterscheinung der somatischen Paarung ist. Nach SVÄRDSON tritt die Paarung während der Anaphase ein und setzt sich bis zur folgenden Prophase fort, also gleicherweise wie es bei *A. fimetarius* der Fall zu sein scheint. Ein ähnlicher Vorgang spielt sich nach GRELL (1945a, b) in den Gonien- und Somazellen von *Culex pipiens* ab, und SMITH (1942) hält das Phänomen auch für charakteristisch für andere

Dipteren. Auch nach SVÄRDSONS Meinung kann es weiter verbreitet sein, als man im allgemeinen glaubt. Er diskutiert auch in seiner erwähnten Arbeit diese theoretisch unleugbar interessante Frage. Meine Befunde an *Aphodius*-Arten stützen wohl seine Auffassungen, sind aber noch zu wenig umfangreich für eine weitere Diskussion.

C. Art-, Gewebe- und Zustandspezifität der Kernstruktur.

Aus der Abb. 4 geht hervor, daß die Art der Variation der Chromocentrenzahl artspezifisch verschieden ist. Ohne jegliche Berücksichtigung des Assoziationsmechanismus kann man sagen, daß Arten mit weniger Chromocentren eine stärkere Neigung zur Chromocentrenbildung haben als solche mit vielen Chromocentren. Demnach wäre jene Neigung bei *A. ater* die stärkste, bei *A. depressus* die schwächste. Solchen Artunterschieden kann man ebensogut bei anderen Zellkategorien begegnen; so sind Chromocentrenzahl und -größe ganz verschieden z. B. in den Vas deferens-Kernen bei *A. foetens* und *A. rufipes*. Ich habe auch in Verbindung mit meinen Spermatogeneseuntersuchungen verschiedene Scarabäidenarten daraufhin verglichen, welcher Eindruck sich von der Heterochromatinmenge bei der Beobachtung der verschiedenen Phasen der Spermatogonien- und Spermatocytenentwicklung ergeben hat (exaktere Messungen konnten nicht angestellt werden), und habe dabei tatsächlich artliche Unterschiede festgestellt. Besonders reich an Heterochromatin sind die Arten *A. fimetarius* und *A. foetens*. Nach den Somazellen beurteilt, weichen die genannten Arten in derselben Richtung von den übrigen, unter sich mehr gleichen Arten ab.

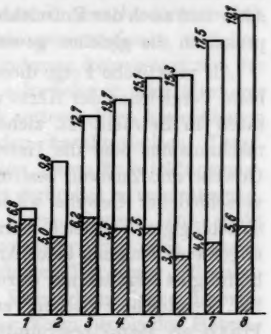

Abb. 8. Vergleich zwischen den Chromocentrenzahlen in Interstitialzellen und in diplotänen Spermatocyten. Die Höhe der Säule entspricht der durchschnittlichen Chromocentrenanzahl der Interstitialzellen, der schraffierte Teil derselben während des Diplotäns. *1 Aphodius ater, 2 A. foetens, 3 A. haemorrhoidalis, 4 A. fimetarius, 5 A. fossor, 6 A. merdarius, 7 A. rufus, 8 A. depressus.*

Es besteht nun eine Vergleichsmöglichkeit auch zwischen den Vereinigungstendenzen in den Interstitialzellen und in den Spermatocyten, denn ich habe bei den letzteren den Mittelwert der diakinetischen Chromocentrenzahlen bestimmt. Wie es Abb. 8 zeigt, sind die artlichen Unterschiede in der Spermatogenese viel unbedeutender als bei den Interstitialzellen. Sie wären vielleicht noch kleiner, wenn es möglich gewesen wäre, genau gleichalte Spermatocyten zu vergleichen. Es ist also klar, daß die Assoziationsneigung in höchstem Maße auch gewebe-

spezifisch ist — eine Tatsache, die sich ebensogut durch Vergleichen des Kernbaues verschiedener Somazellen ein und desselben Individuums feststellen läßt. Dies ist an sich nur zu erwarten, kennt man doch die Gewebespezifität der Kernstruktur in mancher Hinsicht schon lange.

Die Kernstruktur ist außer von der Art und dem Gewebe unzweifelhaft auch vom Zustand der Zelle abhängig. Beispiele dafür sind schon die Phasen der Endomitose selbst. Aber auch andersartigen physiologischen Zuständen der Zelle und des Tieres muß eine Bedeutung beigemessen werden. In welchem Maße solche Faktoren in meinem *Aphodius*-Material wirksam waren, bleibt unentschieden. Jedenfalls sind das Alter und auch der Entwicklungszustand der Hoden bei allen Individuen praktisch die gleichen gewesen[1].

Als praktische Folge dieser Tatsachen ergibt sich die Notwendigkeit, beim Vergleichen der Kern- und Chromocentrenstrukturen alle 3 Spezifitäten in Betracht zu ziehen. Vergleicht man z. B. den Assoziationsmechanismus oder die Heterochromatinmenge bei 2 Arten, so müssen Gewebe und Zustand bestimmt werden, beim diesbezüglichen Vergleich verschiedener Gewebe wiederum Art und Zustand und endlich beim einschlägigen Vergleich verschiedener Zustände Art und Gewebe. Die allgemeine Neigung einer Art zur Heterochromasie und Chromocentrenbildung ist letztlich nur durch Berücksichtigung *aller* Zellarten bestimmbar. Vergleiche zwischen verschiedenen Arten mögen sich jedoch in der Praxis an Hand bestimmter Gewebe durchführen lassen. Zu diesem Zweck sind, wie auch WHITE (1943) bemerkt hat, die ruhenden Somakerne am vorteilhaftesten.

2. Proximale Chromocentrenbildung als eine weitverbreitete cytologische Erscheinung.

Die Neigung der proximalen Chromosomenabschnitte, heteropyknotisch aufzutreten und dabei Assoziationen zu bilden, ist zweifelsohne bei den *Aphodius*-Arten ungewöhnlich groß. Es scheint als käme sie zur Verwirklichung, sobald der Kern beträchtlichere Wachstums- oder überhaupt Produktionssymptome aufweist. In den Interphasen zwischen den späteren Gonienteilungen, wenn das Wachstum von Zelle und Kern gering ist, habe ich bei den Aphodien nur äußerst selten Chromocentren gefunden. Aber das Wachstum der Spermatocyte I bringt schon Voraussetzungen zur Chromocentrenbildung mit sich. Man muß dabei beachten, daß die Spermatocyten bei allen *Aphodius*-Arten eine ganz ungewöhnliche Größe erreichen.

[1] Nach den Beobachtungen von MADLE (1934) hängt der Entwicklungszustand der *Aphodius*-Hoden auch bei gleich alten Individuen in großem Maße von den Nahrungsverhältnissen ab.

Diese Befunde stimmen wohl gut mit der schon eingebürgerten Auffassung überein, daß dem Heterochromatin eine wichtige Rolle bei der Synthese und Wachstumstätigkeit der Zellen beizumessen ist. Es gibt jedoch Arten, z. B. *Melolontha hippocastani* und *Geotrupes stercorarius*, bei denen in der Spermatogenese trotz ganz normalen Spermatocytenwachstums eine intensive Heteropyknose mit Chromocentrenbildung beobachtbar ist[1]. Solche Fälle sprechen vielmehr dafür, daß nicht die Menge, sondern die Qualität oder die Aktivität des Heterochromatins von entscheidender Bedeutung ist.

Die weite Verbreitung der proximalen Chromocentren in den *Aphodius*-Geweben zeugt von einem arteigenen, in verschiedensten Zellkategorien erfüllbaren Bestreben der Centromerenregionen, sich zusammenzuschließen. Dieses Bestreben ist indessen keineswegs nur für die Gattung *Aphodius* allein, sondern in schwächerer oder stärkerer Ausbildung auch für alle bisher cytologisch untersuchten koprophagen und auch für einige phytophage Scarabäiden charakteristisch. Letztlich haben wir es mit einer Eigenschaft zu tun, deren Entwicklungsvoraussetzungen im Tier- und Pflanzenreich weit verbreitet zu sein scheinen. Kennt man doch aus dem Schrifttum (s. hierzu VIRKKI 1951) nicht wenige derartige Assoziationsneigungen proximaler oder auch distaler Chromosomenabschnitte aus sehr verschiedenen Tier- und Pflanzengruppen. Die von Art zu Art feststellbare Verstärkung jener Assoziationen geht meistens Hand in Hand mit der Vermehrung des Heterochromatins, was natürlich zunächst die Annahme aufkommen läßt, daß im Heterochromatin selbst die Ursache des Phänomens liegt (s. z. B. SCHRADER 1941). Fälle, bei denen trotz anscheinend mangelnder oder nur schwacher Heterochromasie eine intensive Chromosomenvereinigung stattfindet (z. B. *Habropogon*, RIBBANDS 1941; *Euchlaena*, LONGLEY 1937), lassen wohl eine kompliziertere Ursache vermuten. Es scheinen, wie auch RIBBANDS (l. c.) annimmt, irgendwelche speziellen Eigenschaften der betreffenden Chromosomenteile mitzuspielen. Ich habe meinerseits früher (VIRKKI l. c.) auf die Möglichkeit hingewiesen, daß die nebeneinander einhergehende Verstärkung der proximalen oder distalen Heterochromasie und der Assoziationsneigungen auf der Duplikation bestimmter, von vornherein mehr oder minder zu Assoziationen neigender Chromomeren beruhen kann. Hält man aber die Assoziationsneigung nicht für eine selbständige Erscheinung, sondern für eine Eigenschaft des Heterochromatins, so kann man sich (wie SMITH 1952) mit der

[1] Man beachte, daß die letztgenannte Art eine der 2 Scarabäiden ist, bei denen deutliche Zusammenschlüsse der Centromerenregionen auch in den Spermatogonien vorkommen. Die zweite ist *Oryctes nasicornis* (auch nach PROWAZEK, 1902), bei welcher das Wachstum der Spermatocyte gering ist und auch die Chromocentrenbildung ausbleibt. — Die Gonien von *M. hippocastani* sind unbekannt.

bekannten Hypothese von MULLER (1918) begnügen, nach der das Heterochromatin vom Euchromatin durch successive, zur „inertness" führende Mutationen entsteht[1]. Dabei ist allerdings zu beachten, daß diese Erklärung auf Grund der bei den Geschlechtschromosomen beobachteten Verhältnisse entwickelt worden ist und darum kaum vorbehaltlos auf die Heterochromasie im allgemeinen angewandt werden kann.

Welches die richtige Erklärung dieser Tatsache auch sein mag, soviel ist klar, daß man es mit einer allgemeinen Differenzierungsmöglichkeit der Chromosomen zu tun hat. Sonst wären die sich weit voneinander im System befindlichen Reihen der Arten, die Heterochromasie und Chromocentrenbildung aufweisen, nicht verständlich. Solchen Faktoren wie der „Klebrigkeit" und dem Ansetzen der heteropyknotischen Teile an die Kernmembran (ohne Polarisation) kann meines Erachtens als Ursachen der Chromocentrenbildung kein großer Wert beigemessen werden, obwohl sie wichtig bei der Erhaltung der fertigen Chromocentren sein können.

Die proximale Heteropyknose scheint besonders weit verbreitet zu sein. Nach LEVAN (1946) gehört der größte Teil aller festgestellten Heteropyknosefälle in den Bereich der proximalen Heteropyknose, und WHITE (1945) hat sogar auf eine solche Möglichkeit hingewiesen, daß sich alle Centromeren vielleicht in einem heteropyknotischen, wennschon kleinen Abschnitt befinden. Von den zahlreichen bekannten Fällen, in

[1] Beruht die ursprüngliche Assoziationstendenz auf der *Homologie* der betreffenden Baueinheiten, so würde die Duplikation zur Entstehung interchromosomal homologer und wahrscheinlich heteropyknotischer Segmente führen. Auch die Resultate der Inertmutationen können in ihrer angenommenen genetischen Wirkungslosigkeit (bzw. Gleichartigkeit) gewissermaßen als homolog angesehen werden. Meines Erachtens gibt es aber auch kaum einen genügenden Grund, dieselbe Möglichkeit wenigstens in bezug auf die heterochromatinisierten Distalenden geradeswegs abzuleugnen. Diese meine Auffassung ist kürzlich von H. SUOMALAINEN (1952) kritisiert worden. Er ist (mit DARLINGTON 1937) erstens der Meinung, daß das abweichende Benehmen der Distalenden durch allgemeine physikalische „Endeffekte" erklärbar sei. Eine *verschiedene* Assoziationsbestrebung der Distalenden bei verschiedenen Arten kann aber nicht durch *allgemeine* Eigenschaften erklärt werden, ohne dabei Verschiedenheiten in der nächsten Umgebung — oder in den Distalenden selbst — vorauszusetzen. Aber als eine solche Verschiedenheit kann, obschon sie das keineswegs muß, z. B. eine verschiedene Heterochromatinisierungsstufe gelten. Anläßlich seiner 2. Bemerkung, daß die Assoziation homologer Distalenden zu Unregelmäßigkeiten in der Konjugation führen müßte, möge nur auf die ziemlich vielen Fälle hingewiesen werden (Literatur z. B. bei VIRKKI op. c.), wo trotz einer strengen Assoziation der Distalenden — sei sie durch Homologie oder durch sonstige Ursachen bedingt — eine ganz normale Konjugation stattfindet. HAYDEN (1925) schildert sogar das Fortschreiten des Konjugationsprozesses bei einer Art (*Phanaeus ignaeus*) mit gleichzeitig ausgebildetem proximalen und distalen Chromocentrum. (Der Fall wäre wohl einer erneuten Untersuchung wert.) Das Gelingen der Konjugation beruht bei solchen Verhältnissen höchstwahrscheinlich auf der aus der vorigen Anaphase stammenden Nachbarlage der Homologen.

denen die proximale Heteropyknose eine Chromocentrenbildung als Begleiterscheinung aufweist, seien hier nur einige, den *Aphodius*-Fällen anscheinend am nächsten stehende erwähnt.

HEITZ (1932) hat bei der Pflanze *Agapanthus* in den somatischen Zellen sternförmige Sammelchromocentren gefunden, die ihrer Entstehungsweise nach den *Aphodius*-Chromocentren sehr ähnlich sind. Eine Verschiedenheit besteht jedoch darin, daß alle Chromosomen zu einem Chromocentrum zusammentreten. Später hat GEITLER (1933) bei demselben Objekt während der Meiose, wenn nicht gerade eine Sammelchromocentrenbildung, so doch bei sämtlichen Chromosomen eine beträchtliche proximale Allocyclie festgestellt.

E. SUOMALAINEN (1946) untersuchte eine Reihe von Schabenarten in bezug auf die Spermatogenese und stellte fest, daß bei *Blabera fusca* die Bivalenten während des Diplotäns mittels ihrer heterochromatischen Proximalabschnitte zu mehreren Chromocentren zusammentreten. Die Chromocentren sind, von der Einarmigkeit und größeren Zahl der Chromosomen abgesehen, denjenigen der *Aphodius*-Arten gleich. Professor SUOMALAINEN hat mir freundlichst seine Präparate geliehen, und ich habe ferner bemerkt, daß auch die Somakerne der Hoden bei der genannten Art mit deutlichen Chromocentren versehen sind. Zwar weisen auch andere Arten seines Materials somatische Chromocentren auf; diese sind indessen unansehnlicher. Es besteht hier also eine Parallele zu den *Aphodius*-Arten: sowohl für die Spermatogenese als auch für die Somazellen ist eine Chromocentrenbildung charakteristisch. Ähnliche Parallelfälle könnten zweifelsohne noch weit mehr zu verzeichnen sein, wenn wir nur etwas von der Somakernstruktur aller derjenigen Arten wüßten, bei denen Chromocentrenbildung während der Meiose vorgelegen hat[1].

Mir ist aus dem Schrifttum kein einziger Fall bekannt, wo der Kernbau demjenigen der Interstitialzellen der *Aphodius*-Arten so ähnlich gewesen wäre wie in den von GEITLER (1941, 1948a) beschriebenen Kernen des Suspensorhaustoriums von *Lupinus polyphyllus*. Die Struktur der Chromocentren scheint bei beiden Fällen gleich und ihre Zahl von der Kerngröße unabhängig zu sein. Die Zahl entspricht jedoch bei *Lupinus* der 2n-Zahl der Chromosomen, während sie bei *Aphodius* der n-Zahl näher steht. Die von GEITLER dargestellten Kerne enthalten verästelte Chromocentren und sind somit am besten als prophasisch zu deuten. Einen endomitotischen Phasenwechsel hat der Verfasser nicht beobachtet, obschon er ihn für wahrscheinlich hält. Hierbei sind

[1] Von der neuesten Literatur auf diesem Gebiet möge nur die Mitteilung GOLDSCHMIDTS (1950) über *Xenopyxis latifrons* erwähnt werden, der zufolge auch die Fische unter den Fällen von meiotischer Chromocentrenbildung vertreten zu sein scheinen. Man beachte hierbei die Befunde SVÄRDSONS (1941) über die Assoziationsneigung der Fischchromosomen.

unzweifelhaft analoge Verhältnisse wie bei *Aphodius* zu erwarten. — Ein grundsätzlich ähnlicher Bau und ähnliches Verhalten bei der Endomitose ist vielleicht ganz gewöhnlich bei solchen Chromocentren, die bei der Endopolyploidisierung ständig wachsen (s. z. B. GEITLER 1941, 1944, 1948a, b; LAUBER 1947).

Ein von MONTALENTI (1949) untersuchter Fall verdient in diesem Zusammenhang besondere Erwähnung. Die Chromocentren sind bei den von ihm beschriebenen somatischen Hodenkernen des Isopoden *Anilocra physodes* äußerlich den *Aphodius*-Chromocentren sehr ähnlich[1].

Seine Interpretationen der Befunde sind indessen wesentlich anders als meine auf die *Aphodius*-Arten begründeten. Die diploiden Vas deferens-Kerne besitzen je ein einziges, sternförmiges Chromocentrum, an dessen Bildung sämtliche Chromosomen beteiligt sind. Höher polyploide Kerne besitzen wiederum mehrere Chromocentren; nach der Annahme MONTALENTIS ist die Tetraploidie durch 2 Chromocentren markiert, die Hexaploidie durch 3, die Oktoploidie durch 4, usw. Die Polyploidisierung käme durch Endomitose zustande, und zwar so, daß sich die Chromocentren (und die Chromosomen in denselben) teilten. Trotz äußerer Ähnlichkeit des Kernbaues wäre also der Entstehungsmechanismus desselben grundlegend verschieden bei *Anilocra* und *Aphodius*.

Ohne auch die durch die Verschiedenheit der Objekte bedingten Unterschiede für unmöglich zu halten, sehe ich mich zu folgenden Bemerkungen genötigt:

1. MONTALENTI hat neben verästelten Chromocentren auch unverästelte gefunden, und namentlich in Kernphasen, in denen nach seinen Ribonukleinsäurebestimmungen eine rege Aktivität herrscht. Der Formwechsel der Chromocentren bei *Anilocra* ist somit demjenigen bei *Aphodius* sehr ähnlich und kann unzweifelhaft auch gleichsinnig gedeutet werden.

2. Solche Polyploidiestufen, wie Hexaploidie, wären wohl verständlich, wenn sich die Chromocentren wirklich unabhängig voneinander teilten. Eine Unabhängigkeit einzelner Elemente von der allgemeinen Phase des Kerns erscheint jedoch zweifelhaft, hat man sich doch daran gewöhnt, den Kern als die kleinste Einheit beim Phasenwechsel und besonders bei den Teilungsphasen zu betrachten[2]. Ferner muß man

[1] In der Meiose weisen die proximalen Abschnitte keine Assoziationen, wohl aber eine abweichende Färbung auf, die jedoch auf anderen, speziellen Eigenschaften, als der Spiralisationsstufe zu beruhen scheint (CALLAN 1940).

[2] Auch GEITLER (1948a) hält den Synchronismus bei der endomitotischen Chromosomenteilung für am wahrscheinlichsten, weist aber auf einige Untersuchungen hin, die für eine entgegengesetzte Sachlage zu sprechen scheinen. Eine begrenzte Asynchronie, wie sie z. B. von STEIN (1942) beschrieben wird, ist an sich nicht auffallend, wohl aber meines Erachtens eine so große Unabhängigkeit einzelner Chromosomengruppen voneinander, wie sie von MONTALENTI angenommen wird.

bezweifeln, ob sich solche kompakten und wahrscheinlich verklebten Heterochromatinklümpchen regelmäßig teilen können. Die zweiteiligen „Teilungsfiguren", wie sie vom Autor geschildert werden, sind meines Erachtens kaum mehr als zufällig zusammengeratene Chromocentren.

3. Mir scheint sicher, daß MONTALENTI seine Auffassungen über den Mechanismus der Polyploidisierung auf Vergleiche zwischen Kernen aus *verschiedenen Geweben* begründet hat. Es ist klar, daß ein solches Verfahren wegen der Gewebespezifität der Kernstruktur leicht irreführen kann.

Nach dem Obengesagten ist es mir schwer, von der Richtigkeit der Erklärungsweise MONTALENTIS überzeugt zu werden, solange nicht sicherere Gründe für sie beigebracht werden können.

Einigen gründlicher geklärten Fällen, in denen die Kernstruktur prinzipiell derselben der *Aphodius*-Arten nahe steht, begegnen wir unter den Dipteren. So findet z. B. in den Darmzellen der *Culex*-Larve nach BERGER (1938a, b) und GRELL (1945a, b) eine endomitotische Polyploidisierung[1] statt, wobei die Tochterchromosomen ziemlich eng zusammenhängen bleiben. Wegen einer somatischen Konjugation, die auch bei diesem Dipter die Regel zu sein scheint, enthalten die so entstandenen Bündel alle Abkömmlinge beider Homologen, also gleicherweise wie dies wenigstens für die Interstitialzellen von *A. foetens* anzunehmen ist. Wie auch LORZ (1947) betont, ist der Kernbau der Darmzellen der *Culex*-Larve demjenigen der Speicheldrüsenzellen mancher Dipteren nahe verwandt. Die Riesenchromosomen sind tatsächlich Chromosomenbündel, die ihre Entstehung der ausgebliebenen Trennung der Tochterchromosomen bei den Endomitosen verdanken; sie werden auch aus somatisch konjugierten Homologenpaaren aufgebaut. Bei *Drosophila* haben die Riesenchromosomen dazu durch Zusammentreten ihrer proximalen Abschnitte sog. Chromocentren gebildet, die den *Aphodius*-Chromocentren prinzipiell weitgehend ähnlich sind. Wie bei den *Aphodius*-Arten, tritt auch bei den Dipteren die Assoziationsneigung der Chromosomen sowohl in der Meiose (vielfach Bivalenten ohne Chiasmata beim ♂) als auch im Soma hervor (vgl. BERGER 1936, MAKINO 1940).

Es gibt jedoch einige Unterschiede. Während die somatische Konjugation stets in den Urspeicheldrüsenzellen von *Drosophila* und überhaupt in den Interphasekernen der Dipteren vorherrscht, scheint sie in

[1] Einige Autoren und besonders GRELL wollen den Ausdruck *Endomitose* für Fälle mit vollständigem Phasenwechsel, wie ihn GEITLER ursprünglich beschrieben hat, vorbehalten. Mir scheint, daß der Gewinn an Genauigkeit dabei den Verlust an Bequemlichkeit bei der Benennung nicht aufzuwiegen vermag. Es gibt ja bei den inneren Teilungsprozessen kaum tiefere als lediglich Gradunterschiede zwischen den Fällen mit vollständigem, mehr oder minder unvollständigem und fehlendem Spiralisationscyclus, weshalb eine gemeinsame Benennung für die Erscheinung erforderlich ist.

den Interstitialzellen der *Aphodius*-Arten bei manchen Homologenpaaren auszubleiben. Während ferner die Proximalteile aller Chromosomen bei *Drosophila* zu einem einzigen Chromocentrum zusammentraten, bleiben bei *Aphodius* die Homologengruppen bzw. Chromosomen insbesondere in den Interstitialzellen größtenteils frei bestehen. Der 3. Unterschied besteht darin, daß die Speicheldrüsenchromosomen gänzlich halbnukleinisiert sind, während bei *Aphodius* die proximalen und dazu meistens die subproximalen Chromosomenabschnitte in einem nukleinisierten Zustand verbleiben. Die Unterschiede sind keineswegs bedeutend und werden dazu schon durch Verhältnisse in verschiedenen Zellkategorien der betreffenden Arten überbrückt. Die Verschiedenheiten können von mehr quantitativer oder mehr qualitativer Natur sein, auf jeden Fall bleibt das allgemeine Bauprinzip der Chromocentren bei beiden Gattungen gleich: endomitotische Vervielfachung der (somatisch konjugierten) Chromosomen mit einer gleichzeitigen Assoziation der heteropyknotischen Centromerenregionen. Die weite Verbreitung der proximalen Chromocentren im Tier- und Pflanzenreich läßt meines Erachtens wohl auch weiterhin gleichartige Befunde in bezug auf das Verhalten der Chromozentren bei der Polyploidisierung erwarten.

Zusammenfassung.

Es wurde der Kernbau somatischer Hodenzellen bei 15 *Aphodius*-Arten analysiert. Als günstigste Objekte erwiesen sich die interstitiellen Hodenzellen, besonders der Arten *A. foetens* und *A. fimetarius*. — Die wichtigsten auf die Interstitialzellen bezüglichen Befunde sind:

1. Das Volumen der Kerne (nebst der Zellen) variiert in sehr weiten Grenzen. Es bestehen unzweifelhaft mehrere Polyploidieklassen.

2. Die Kerne enthalten Chromocentren, deren Formwechsel durch den Phasenwechsel der Endomitose gedeutet werden kann, und zwar sind Kerne mit verästelten Chromocentren als endoprophasisch bzw. endometaphasisch, diejenigen mit unverästelten als endointerphasisch anzusehen. Endoanaphasen sind dagegen nicht beobachtet worden.

3. Die Zahl der Chromocentren des Kerns bleibt im allgemeinen unterhalb 20 (der 2n-Zahl der Arten) und ist praktisch gesehen vom Volumen der Kerne unabhängig. Der Mittelwert liegt gewöhnlich zwischen 10 und 20, näher der ersteren Zahl (der Haploidzahl).

4. Die Chromocentren sind durchschnittlich in den größeren Kernen größer als in den kleineren.

Nach diesen und den früher (VIRKKI 1951) bei der Spermatogenese gemachten Beobachtungen kann geschlossen werden, daß die Chromocentren Vereinigungen proximaler, heteropyknotischer Chromosomenabschnitte darstellen, und daß die bei der Endomitose entstehenden Tochterchromosomen im allgemeinen an den Chromocentren verbunden

bleiben. Wenigstens bei *A. foetens* spricht die Variation der Chromocentrenzahl für das Vorhandensein einer somatischen Konjugation im Beginn der endomitotischen Polyploidisierung. — Nach alledem stünden die *Aphodius*-Chromocentren grundsätzlich den *Drosophila*-Chromocentren nahe.

Auch bei den anderen Somakernen des Hodens begegnet man gleichartigen Chromocentren, deren Zahl jedoch infolge einer strengeren Vereinigungsneigung der Chromosomen viel kleiner bleibt.

Diese Untersuchung wurde ausgeführt mit Unterstützung des sog. Akademiehilfsgeldes des Finnischen Staates.

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Chromosoma, Bd. 6, S. 33—44 (1953).

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THE REGIONS OF SPECIAL CYCLE OF DIVISION OF AGAPANTHUS CHROMOSOMES.

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With 17 figures in the text.

(Eingegangen am 15. Mai 1953.)

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I. Introduction.

During the processes of mitosis and meiosis the division of the chromosome body does not take place at the same time in all its parts. Certain regions of the chromosome can be seen to be divided earlier than others.

At the early prophase of mitosis the chromosome threads may be seen to be double, and until metaphase the chromatids are held together less closely elsewhere than at the kinetochore. The end of metaphase of mitosis is marked by a change in the kinetochore. At this point the two sister chromatids which have lain closest together now separate. The only part of the chromosome that is considered to divide at metaphase of mitosis is the kinetochore, the chromosome arms being supposed to have divided as early as prophase (DARLINGTON 1937 and others).

During meiosis each chromosome, from early diplotene onwards may be seen to consist of two sister chromatids. These chromatids remain in close association throughout most part of the first division and become widely separated — in the region of the arms — at prophase and metaphase of the second division. Until the end of metaphase II the chromatids of each chromosome are considered to be held together only at their kinetochores (DARLINGTON 1937 and others).

The view held at present by most cytologists is that the localized kinetochore or centromere exhibits two properties: the property of active mobility on the spindle and the property of a special cycle of division. Whereas the chromosome arms of each chromosome are

considered to be divided from the prophase of mitosis and from the diplotene stage, the kinetochore is supposed to remain undivided until the end of metaphase of mitosis and until the end of metaphase II of meiosis (*cf.* LIMA-DE-FARIA 1949b).

II. Material and Methods.

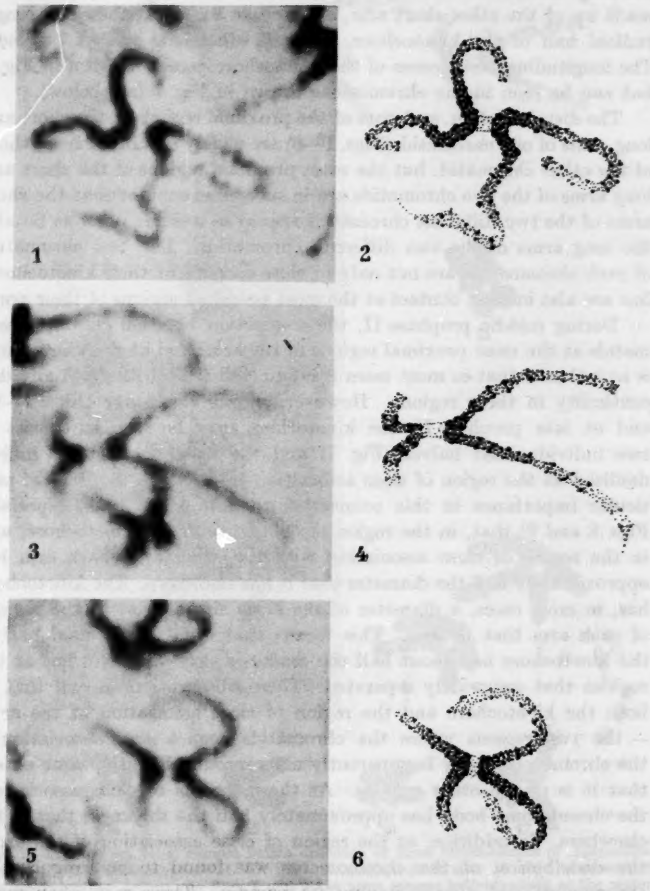
The plants used in this study were specimens of *Agapanthus umbellatus* growing in the Botanical Garden of the University of Lund. The material was fixed in acetic-alcohol 1:4 for 4—6 hrs., transferred to 95% alcohol, where it was left over night, being stored after this time in 70% alcohol. The preparations were made after a few days according to the squash technique used for pachytene chromosomes of rye (LIMA-DE-FARIA 1948 and 1952). The stain used was iron-aceto-carmine; the mounting medium was Canada balsam. No pre-treatment was used. The observations described below were made in a large number of cells (more than one hundred) belonging to two preparations in which staining and squashing of the chromosomes were particularly good.

III. Observations.

In *Agapanthus umbellatus* interphase is a stage that can be well separated from prophase II. At interphase the chromosomes are not fully individualized and pro-chromosomes may be observed in the nucleus. At prophase II — which is a relatively long stage — the chromosomes are individualized and appear deeply stained on both sides of the kinetochore. The staining intensity of the chromosome arms decreases gradually towards the ends, where the chromosomes appear very faintly stained (Figs. 1—9). The kinetochore has in most cases the same intensity of stain as the ends of the chromosome. This gradient in staining intensity — which originates on both sides of the kinetochore — is accompanied by a gradient in chromomere size. The chromomeres can not be so well analysed at this stage as at pachytene but are however quite distinct in the chromosome arms. In the kinetochore no chromomeres could be observed. The chromomeres are large on both sides of the kinetochore and decrease gradually in size as one approaches the ends (Figs. 1—9). Moreover, this decrease in both staining intensity and chromomere size is related to the arm length. In short arms this decrease is more sudden than in long arms, this phenomenon being characteristic of all the 15 chromosomes ($2n = 30$) of *Agapanthus umbellatus*.

The majority of the chromosomes of *A. umbellatus* have arms of different size. This feature makes it possible to identify with certainty at prophase II, which part of a chromosome belongs to one chromatid and which part belongs to the other chromatid. One short arm with one long arm make part of a chromatid, and the other short arm and long arm make part of the other chromatid. The chromosome will divide at the beginning of anaphase II, into two daughter chromosomes, through a plane which passes between both short arms and both long

arms. One short arm and one long arm will pass to one pole, the other short arm and long arm will pass to the opposite pole.



Figs. 1—6. Photomicrographs of three different chromosomes of *Agapanthus umbellatus* at middle prophase II and their respective camera lucida drawings. The kinetochore is the weakly stained region found between the short and the long arms. One short arm with one long arm make part of a chromatid and the other short arm and long arm make part of the other chromatid. — 2500 ×.

In the chromosome represented in Figs. 1 and 2 it can be seen that one of the chromatids of this chromosome is made up of the short arm,

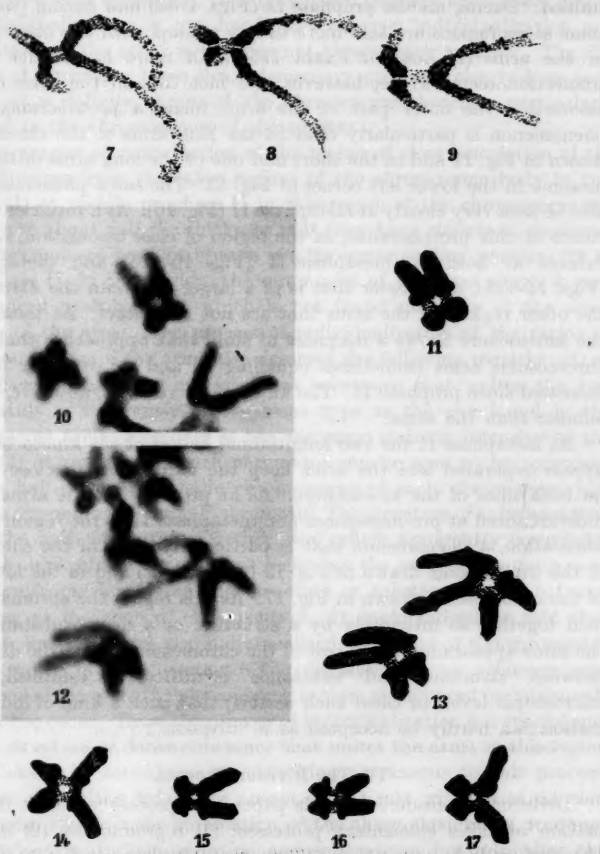
situated at 12 o'clock, the longitudinal half of the kinetochore (the kinetochore is the weakly stained region found between the short and the long arms) and the long arm at 3 o'clock. The other chromatid is made up of the other short arm, situated at 9 o'clock, the other longitudinal half of the kinetochore, and the other long arm at 7 o'clock. The longitudinal doubleness of the kinetochore cannot be seen in Fig. 2 but can be seen in the chromosome drawn in Fig. 7 (see below).

The distal, median, and part of the proximal regions of the short and long arms of one chromatid (Figs. 1—6) are widely separated from those of the other chromatid, but the most proximal regions of the short and long arms of the two chromatids are in such close contact that the short arms of the two different chromatids appear as a single unit; as do also the long arms of the two different chromatids. The two chromatids of each chromosome are not only in close contact at their kinetochores but are also in close contact at the most proximal regions of their arms.

During middle prophase II, the connection between the two chromatids at the most proximal regions of the arms and at the kinetochore is so intimate that in most cases it is impossible to distinguish any discontinuity in these regions. However, during this stage (Figs. 1—9), and at late prophase II, the kinetochore may be seen to consist of two individualized halves (Fig. 7) and the arms appear also individualized at the region of close association (Figs. 8 and 9). It is of particular importance in this connection to note (Figs. 1—9, especially Figs. 8 and 9) that, in the region of contact with the kinetochore, and in the region of close association with the other arm, each arm has approximately half the diameter that it has elsewhere. The kinetochore has, in most cases, a diameter of the same size as that of the regions of each arm that lie free. This means that each longitudinal half of the kinetochore has about half the diameter that each arm has at the regions that are widely separated. These observations reveal that at both the kinetochore and the region of close association of the arms — the two regions where the chromatids keep a close association — the chromosome body is apparently not reproduced to the same extent that it is in the other regions. At these regions of close association, the chromosome body has approximately half the thickness that it has elsewhere. In addition, at the region of close association of the arms, the distribution of the chromomeres was found to be irregular — a chromomere not always having its partner. These two phenomena show that, at this region, the reproduction of the chromosome body has not taken place in the same way as at the other regions of the arms which lie widely separated.

At pro-metaphase II (Figs. 10—13) the two longitudinal halves of the kinetochore may be seen to be already separated, but the two chromatids are still held together at the region of the arms situated on

both sides of the kinetochore. The kinetochore has now become nearly as deeply stained as the chromosome arms, a fact that makes it still



Figs. 7—17. — Figs. 7—9, camera lucida drawings of three chromosomes of *Agapanthus umbellatus* at middle prophase II. In Fig. 7 the kinetochore may be seen to consist of two individualized halves. In Figs. 8 and 9 the arms appear individualized at the region of close association (in Fig. 9 only the short arms). — Figs. 10—13, pro-metaphase II. The two longitudinal halves of the kinetochore are already separated. — Figs. 10 and 12 photomicrographs of the chromosomes drawn in Figs. 11 and 13 respectively (also camera lucida drawings). — Figs. 14—17, camera lucida drawings of four different chromosomes at metaphase II. The two sister chromatids of each chromosome are held together at the most proximal regions of the arms and not at the kinetochore. — 2250 ×.

easier to check its separation into its two halves, as may be seen in the photomicrographs (Figs. 10 and 12) and their respective camera

lucida drawings (Figs. 11 and 13). The proximal regions of the arms, where the region of close association is situated, are particularly deeply stained. During middle prophase II (Figs. 1—9) and during pachytene these same regions are also more deeply stained than the other regions of the arms (*cf.* LIMA-DE-FARIA 1950). Of more importance in this connection seems to be, however, the fact that in the zone of close association the outer part of the arms forms a protuberance. This phenomenon is particularly clear in the long arms of the chromosome drawn in Fig. 11 and in the short and one of the long arms of the chromosome in the lower left corner of Fig. 13. The same phenomenon can also be seen very clearly at metaphase II (Fig. 16). As a result of the existence of this protuberance, in the region of close association, the arms exhibit at both pro-metaphase II (Figs. 10—13) and metaphase II (Figs. 14—17), a diameter that is of a larger size than the diameter of the other regions of the arms that are not in contact. At these stages the kinetochore shows a deepness of stain that approaches that of the chromosome arms (sometimes equalling it) and its diameter has also increased since prophase II. The kinetochore remains, however, slightly thinner than the arms.

At metaphase II the two longitudinal halves of the kinetochore still appear separated and the arms keep the chromatids in close contact on both sides of the kinetochore. As at prophase II, the arms appear individualized at pro-metaphase and metaphase II at the region of close association, a phenomenon that is particularly clear in the short arms of the chromosome drawn in Fig. 13 (upper right) and in the long arms of the chromosome drawn in Fig. 17. At this region the chromatids are held together so intimately by a structure or a dense substance with the same appearance as the rest of the chromosome body (the difference between "structure" and "substance" is difficult to establish, at the microscopic level, in cases such as this) that such a kind of individualization can hardly be accepted as a "division".

IV. The Division Process.

Division is considered in this paper as a process which is the summation of three elementary processes: (1) reproduction, (2) individualization and (3) separation. When these three processes have taken place in a chromosome region it is said that this region has divided. Each of these three elementary processes has sometimes been taken as synonymous with division by various workers, the result being a regrettable confusion. The two first processes, reproduction and individualization, are necessary steps in the accomplishment of the third and final process of separation. At the same time it is not always easy, or even possible, to know at which stage the processes of reproduction

and individualization take place in the various chromosome regions. The only process of the three which can be checked with any certainty is the process of separation. The distinction between the various grades of individualization on one hand, and between individualization and separation on the other is likewise not always easy to draw. The difficulty of separating these three elementary processes clearly from each other, at a definite region of the chromosome, becomes particularly marked in the chromosomes of *Agapanthus*.

The process of reproduction at the region of close association of the arms diverges from the other regions of the chromosome body in two ways: (1) at middle prophase II in this region of the chromosome the arms have about half the thickness that they have elsewhere, moreover each chromomere has not always got its corresponding partner, (2) at metaphase II, in this region, the outer part of the arms shows a very pronounced protuberance, which is not found normally at the other regions of the arms. The process of individualization at the region of close association of the arms also deserves the following remarks: (1) at metaphase II the structure or dense substance that unites the two chromatids is apparently of the same type as the one found in the chromosome arms; moreover it has the same staining intensity as the arms, (2) at prophase II each chromatid can be seen to be composed of two half-chromatids — the chromomeres of each chromomere pair each corresponding to one half-chromatid. The structure or substance that keeps the half-chromatids together, and which apparently constitutes the main bulk of the chromosome body has the same appearance — at the microscopic level — as the structure or substance found between the arms at the zone of close contact. It could indeed be said that the half-chromatids are also individualized, as is said of the chromatids at the region of close contact. The distinction is an arbitrary one, and in one of the cases the phenomenon is more pronounced (or advanced) than in the other. Thus, the process of individualization is a special one, for the structure or dense substance that unites the arms at this region cannot easily be considered as something extraneous to this process.

These difficulties led me to accept as the sole criterion of division the process which is the summation of the three elementary processes of reproduction, individualization and separation; for only after the three are accomplished may these difficulties be removed. To take one of these elementary processes as sole criterion of division is to oversimplify the process in question. This leads to an under-estimation of its complexity and in this way directs research away from a deeper analysis of its mechanism.

The various steps in the division process of the various chromosome regions may be summarized as follows:

(1) Middle and late prophase II.

(a) Proximal (region of close association excepted), median and distal regions of the arms. Reproduced, individualized and separated.

(b) Region of close association (chromosome arms). The reproduction has apparently not progressed so far as in region (a). Individualized. Not separated.

(c) Kinetochore. The reproduction seems not to have progressed to the same extent as in region (a). Individualized. Not separated.

(2) Pro-metaphase and metaphase II.

(a) Proximal (region of close association excepted), median and distal regions of the arms. As before.

(b) Region of close association (chromosome arms). Reproduction apparently accomplished; formation of protuberances. Individualized. Not separated.

(c) Kinetochore. Reproduction apparently accomplished. Individualized. Separated.

At prophase II of meiosis part of the proximal, the median and the distal regions of the arms are divided. The kinetochore and the most proximal region of the arms are not divided. At metaphase II of meiosis part of the proximal, the median and the distal regions of the arms, and the kinetochore are divided. The most proximal region of the arms is not divided. The division of each chromosome into two daughter chromosomes is accomplished at the beginning of anaphase II when the two sister chromatids separate at the most proximal region of the arms.

V. Discussion.

The phenomenon just described could be easily studied in the chromosomes of *A. umbellatus* owing to four main reasons:

(1) At prophase and metaphase of mitosis the two sister chromatids of each chromosome are normally closely associated throughout most of their length. The observations here reported were made at prophase and metaphase II. At these stages the two sister chromatids of each chromosome are, in most materials, well separated from each other throughout most of their length.

(2) A large number of the chromosomes of *A. umbellatus* have arms of different size. This allows one to determine with certainty the plane of division of the chromosome at anaphase II. When a chromosome has arms of equal size such a determination is not easily made.

(3) Prophase II, in *A. umbellatus*, is a relatively long stage. The large size of the chromosomes also contributes to its ease of study in this species.

(4) This study was carried out with preparations made of squashes of P.M.C.'s. In sections, the thickness of the cytoplasm and the entanglement of the chromosomes may lead to uncertainties. In these

preparations the chromosomes lie almost in a single plane, which greatly facilitates the observations. Moreover, the pressure used in making the preparations would only tend to separate the sister chromatids from each other. As after such pressure the chromatids are still kept together so strongly at the regions described above, the use of this method appears to be particularly favourable for such a study.

At prophase and metaphase of mitosis it is not to be expected that this phenomenon could be easily studied, owing to the close association of the two chromatids of each chromosome throughout most of their length. However, if after the use of a particular treatment the chromatids were found to be widely separated from each other for most of their length, this phenomenon might be analysed also at mitosis. This is the case in materials treated with colchicine and other chemicals where X-shaped c-pairs appear. The author has already obtained evidence from the study of preparations of root tips of *Allium Cepa*, treated with colchicine, that the phenomenon here described at meiosis for *A. umbellatus* may be studied at mitosis after such a treatment.

In each chromosome of *A. umbellatus* there are three regions with distinct cycles of division: (I) the proximal (the region of close association excepted) median and distal regions of the arms which are divided since early prophase II, at least, (II) the kinetochore which appears to be divided at pro-metaphase II, and (III) the most proximal region of the arms which only divides at the beginning of anaphase II. The fact that on both sides of the kinetochore the arms remain in close association, could lead one to think that this condition might impede the division of the kinetochore. It may be that this situation may interfere in a limiting manner on the cycle of division of the kinetochore, but it can be seen that the kinetochore has a cycle of division of its own distinct from the other two regions of the arms to which reference has just been made. At middle prophase II the kinetochore has about the same staining intensity as have the chromosome ends, yet each longitudinal half of the kinetochore has about half the thickness of each end. At pro-metaphase II the kinetochore may be seen to be already divided while the most proximal part of the arms does not divide until the beginning of anaphase II.

The property of keeping the sister chromatids together until the end of metaphase II has been earlier attributed solely to the kinetochore. The present observations reveal, however, that if the kinetochore plays a role in this process, it appears to be an indirect one, and that the region responsible for such a phenomenon is situated in the arms. Such a finding has several implications.

T-ends, the ends of some chromosomes of rye that at meiosis exhibit the property of active mobility on the spindle, were found to differ from localized kinetochores in two main properties. They lacked the property

of holding together the sister chromatids until the end of metaphase II and the phenomenon was only exhibited at meiosis (*cf.* LIMA-DE-FARIA 1949b). The present finding, that the region responsible for holding together the sister chromatids until the end of metaphase II appears to be situated in the arms, and not in the kinetochore, reveals that kinetochores and t-ends are regions of much more similar function than was supposed before.

In rye and other species (MÜNTZING 1946, ÖSTERGREN 1947, HÅKANSSON 1948 and BOSEMARK 1950) it was found that certain types of B chromosomes exhibit the phenomenon of non-disjunction at the first pollen mitosis (also in rye at the corresponding stage on the female side). At anaphase of the first pollen mitosis the two sister chromatids of these B chromosome types are held together at definite regions of the arms. As a result of this phenomenon the two sister chromatids of a chromosome, instead of separating normally at anaphase, often pass united to the anaphase group which will later become the generative nucleus. As a result of a comparative study of this phenomenon it was pointed out (LIMA-DE-FARIA 1949b) that the regions responsible for the phenomenon of non-disjunction represented an example of regions of the arms exhibiting the property of a special cycle of division — in this case, the property of holding together the two sister chromatids of a chromosome at least during anaphase of the first pollen mitosis. At that time the property of the chromosome of holding together the two sister chromatids until the end of metaphase II was supposed to be only exhibited by the kinetochore and not by the arms. This was a serious objection to considering the two phenomena as closely related. Now that the region of the chromosome primarily responsible for holding together the sister chromatids until the end of metaphase II is found to be situated in the arms, and not in the kinetochore, this obstacle is removed, and the two phenomena appear as intimately related.

SCHRADER (1939) found out that the kinetochore was individualized into two longitudinal halves already in diakinesis, and that at metaphase and anaphase I of meiosis each chromosome showed two spindle spherules distinctly separated. Owing to the fact that the spindle spherules were already divided at these stages, and the two sister chromatids were kept together until the end of metaphase II, SCHRADER was led to conclude that the two properties of the kinetochore: active mobility on the spindle and the holding together of the chromatids were carried out by two distinct structural units. This was an important conclusion. He suggested, however, that the binding element of the kinetochore could be considered to be the cup or commissural mass. Owing to the fact that the commissural region has been seen to appear double at metaphase by KOSLOV (1937), the writer was led (LIMA-DE-

FARIA 1949a) to ascribe the property of a special cycle of division to the most internal region of the kinetochore of the pachytene chromosomes of rye. It may still be that this most internal region of the kinetochore — the deeply stained fibrilla pair found between the chromomere pairs — may be directly connected with the special cycle of division of the kinetochore, as the kinetochore has a cycle of division of its own. It is, however, now clear, at least in *A. umbellatus*, that the region of the chromosome that appears to be primarily responsible for keeping the sister chromatids together until the end of metaphase II is the most proximal region of the arms. None of the regions of the kinetochore of the chromosomes of *A. umbellatus* appears to take direct part in this process.

In *Ulophysema öresundense* (MELANDER 1950) it was found that at early prophase of mitosis the sister chromatids (or future daughter chromosomes as they may also be called) of each chromosome are widely separated for most of their length. The sister chromatids of each chromosome are only kept together at the *distal* region of the arms, where the chromosome body appears to be particularly thick (the "collasome"). At the other extremity where the kinetochore is situated the chromatids lie widely separated. The observations made here in *Agapanthus* throw new light on the abnormal behaviour of these chromosomes. The kinetochore appears in *Ulophysema öresundense* to have no direct connection with the phenomenon of holding the chromatids together until the end of metaphase of mitosis. This observation is in good agreement with the results obtained here in *Agapanthus*. In *Ulophysema* the region of the chromosome that holds the sister chromatids together is the most distal region of the arms, whereas in *Agapanthus* this region is the most proximal. The binding element of the chromatids is, however, in both cases, situated in the arms, the difference in the two cases appearing to be mostly one of position. In one case the region in question is situated close to the kinetochore, in the other far away from it. The regions of the arms of the B chromosome types which exhibit the property of a special cycle of division would represent an intermediate condition between the *Agapanthus* and the *Ulophysema* cases. In the B chromosome types these regions of special cycle of division are situated either at the ends or in the middle of the arms.

The fact that the region of close association of the sister chromatids varies so widely in position in the arms, and at the same time seems to be situated in most cases in the close vicinity of the kinetochore leads one to think that possibly the property of holding the sister chromatids together until the end of metaphase II may also be exhibited in other cases by the kinetochore itself.

Summary.

(1) In the chromosomes of *Agapanthus umbellatus* the kinetochore can be seen to be divided as early as pro-metaphase II. At this stage, the two longitudinal halves of the kinetochore are individualized and separated from each other. The region of the chromosome body that appears to be responsible for holding together the two sister chromatids of each chromosome until the beginning of anaphase II is the most proximal region of the arms and not the kinetochore.

(2) At the second division of meiosis, three regions with distinct cycles of division may be distinguished in the chromosomes of this species: (1) the kinetochore which can be seen to be divided from pro-metaphase II, (2) the most proximal regions of the arms which only divide at the beginning of anaphase II and (3) the rest of the chromosome arms which appears to be divided from early prophase II. The concept of division is revised.

(3) The kinetochore has its own cycle of division but does not appear to take direct part in holding together the two sister chromatids of each chromosome until the beginning of anaphase II. The fact that this last property appears to be situated in the arms has several implications. The behaviour of t-ends and of the regions of the B chromosome types responsible for the phenomenon of non-disjunction can be better understood in the light of this finding. In addition, the particular type of division of the mitotic chromosomes of *Ulophysema öresundense* can be more easily interpreted.

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SOME CYTOLOGICAL ASPECTS OF ANTAGONISM IN SYNTHESIS OF NUCLEIC ACID*.

By

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With 17 figures in the text.

(Eingegangen am 6. Juni 1953.)

Introduction.

Biological antagonism in enzyme systems has come to be recognized as an interference in the enzyme-substrate relationship by some compound whose molecular structure resembles that of the substrate. When QUASTEL and WOOLDRIDGE (1928) showed that the action of succinic dehydrogenase is inhibited by malonic acid they demonstrated the competitive nature of succinic and malonic acids in relation to the enzyme. The inhibition of bacterial growth by sulfanilamide was explained by WOODS (1940) as owing to competition with p-aminobenzoid acid; its effects could be reversed by adding more p-aminobenzoic acid to the medium. This study extended the possibility of exploring analogue and metabolite-enzyme relationship to a living cell. It showed that the metabolite whose utilization was interrupted could be specified among the multiplicity of compounds within a cell. Many application of this reasoning have been outlined by WOOLLEY (1946 and 1952).

SNELL and MITCHELL (1941) found that adenine, guanine, thymine, and uracil are necessary for growth of various forms of *Lactobacillus casei* and that even when these bases of nucleic acid are not essential their addition to the culture medium could be stimulatory. An interference in the metabolism of these compounds might result in decreased growth. WOOLLEY (1944), KIDDER and DEWEY (1949a and b) and HITCHINGS, ELION, FALCO, RUSSELL, and VAN DER WERFF (1950) have shown that there is an antagonism between purine and pyrimidine components of nucleic acid and certain structural analogues. Presumably one area where competition could occur is in the synthesis of nucleic acid.

* Contribution from the Program in Cytology, Department of Botany University of Wisconsin, Madison, supported in part by grants to Dr. C. LEONARD HUSKINS from the American Cancer Society, Rockefeller Foundation, and Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation.

FURST, ROLL, and BROWN (1950) found that isotope-labelled dietary adenine could later be detected in both PNA (pentose nucleic acid) and DNA (desoxypentose nucleic acid) of the liver and total viscera of rats. The amount of labelled adenine which had been incorporated in DNA was roughly proportional to the number of mitoses. One analogue of nucleic acid purines, 6-diamino purine, inhibits the growth of *Lactobacillus casei*; HITCHINGS, ELION, VAN DER WERFF, and FALCO (1947) found that its inhibition is overcome competitively by adenine. BENDICH and BROWN (1948) found that this same compound can serve as the precursor of nucleic acid guanine. MITCHELL, SKIPPER, and BENNETT (1950) detected another purine analogue, 8-azzaguanine, in PNA extracted from the viscera of mice which had been injected with a solution of it. Apparently adenine and some of its analogues are incorporated directly into nucleic acid.

The action of analogues of other bases of nucleic acid may not be so straightforward. PLENTL and SCHOENHEIMER (1944) demonstrated that guanine, thymine, and uracil as free bases are not precursors of nucleic acid. BROWN (1950) added cytosine to this list and suggested a pathway by which adenine was transformed to guanine. HAMMERSTEN, REICHARD, and SALUSTE (1950) showed that cytidine can serve as a precursor of the pyrimidines of both PNA and DNA. It has been suggested that after linkage to ribose, decarboxylation, and attachment of the appropriate side groups, orotic acid can serve as a precursor of pyrimidine ribosides. ARVIDSON, ELIASSON, HAMMERSTEN, REICHARD, VON UBISCH, and BERGSTROM (1949) demonstrated that isotopic N in orotic acid could be detected later in uridine and cytidine. Analogues of guanine and any of the pyrimidine bases in nucleic acid must therefore exert any disruptive effect on synthesis of nucleic acid in a more indirect way than do analogues of adenine. Cytological aspects of response to treatment with analogues of purine and pyrimidine components of nucleic acid might be rather diverse and interesting.

Material and methods.

Benzimidazole and 5-aminouracil (both obtained from Distillation Products Industries) were used in this investigation. Adenine sulfate, folic acid (both obtained from the Nutritional Biochemicals Corporation) and thymine (from Schwarz Laboratories, Inc.) were used in reversing the effects of the antimetabolites. Solutions of benzimidazole (150, 300, and 600 p.p.m.), 5-aminouracil (31, 50, 63, 125, 250, and 500 p.p.m.), adenine sulfate (800 p.p.m.), folic acid (5 p.p.m.), thymine (50 p.p.m.), and three mixtures (adenine sulfate 800 p.p.m. and benzimidazole 300 p.p.m.; thymine 50 p.p.m. and 5-aminouracil 50 p.p.m.; and the last combination plus folic acid 5 p.p.m.) were prepared in aerated distilled (in glass) water and their p_H adjusted to 5.8 ± 0.3 with dilute hydrochloric acid.

Bulbs of the common onion, *Allium cepa*, were placed on shell vials (19×65 mm) which had been previously cleaned with acid and filled with aerated distilled (in

glass) water. These were maintained at 20.0± 0.5° C. The water in each vial was replaced every 24 hrs. with fresh water which had been aerated by shaking vigorously. After 48 hrs. the roots were of sufficient length to begin treatments. To remove excessive variation only bulbs with roots of length averaging between 10 and 20 mm and with intrabulb variation in root length of no more than 10 mm were used (see PATAU and PATIL, 1951). Bulbs with extremely thick or thin roots were excluded. The remaining bulbs were then arranged in length-classes according to decreasing length of roots and treatments were assigned at random to each bulb of the various length classes until each treatment had 5 replicates, one within a length class. A single root was taken at random from each bulb of a treatment at 0, 6, 12, and 24 hrs. and fixed in 3:1 alcohol-acetic mixture. Squash-preparations were made from a small portion of the meristem just back of the root cap after the whole root was stained by the Feulgen method. Determinations of percentage of nuclei undergoing division were made by classifying, as mitotic or energic, the nuclei of the first 500 cells encountered while moving the mechanical stage from a starting point taken at random within the mass of cells.

Determinations of the relative content of Feulgen-stained material of nuclei in treated and untreated root meristems were made by means of a microphotometer. The apparatus used was essentially like that described by RIS and MIRSKY (1949) except that a camera lucida-like attachment was placed just above the eyepiece. Outlines of the nuclei being studied could then be made and the areas of their cross sections determined by a planimeter.

The methodological procedures outlined by RIS and MIRSKY (1949) and SWIFT (1950a) for animal tissues and particularly those which SWIFT (1950b) and NELSON (1951) used for plant material were adopted. A minor variation was made to ensure that roots in different treatments had been handled similarly. Three roots, each from a different bulb (each bulb had a different treatment but belonged to the same length class), were fixed together in the same vial. Because the roots from different treatments were cut at different lengths, they could be distinguished and could be processed together until they were squashed on separate slides. The fixative used in this part of the study was 20% formaldehyde solution neutralized with calcium carbonate. After 3 hrs. fixation the roots were washed overnight in running water, hydrolyzed in normal HCl for 14 min at 60° C, and stained by the Feulgen method for $^1/_2$ to 2 hrs. The procedure of making squash-preparations was the same as described earlier. From each slide made from one root meristem thirty interphase nuclei were taken at random and their content of Feulgen stain measured. A few early prophases and late telophases also were selected for measurement. The extinction value of each nucleus (E) was multiplied by its cross sectional area (A) to give in arbitrary units a value which is an equivalent of the DNA content of the nucleus. All values were converted to logarithms before plotting.

Results and observations.

The results of preliminary experiments assessing the reduction in number of nuclear divisions brought about by different concentrations of benzimidazole and 5-aminouracil are presented in Table 1. Each value in this table is the average of the percentages of dividing nuclei in two roots from different bulbs. There is a relatively much smaller percentage of nuclei in division in roots treated with solutions containing benzimidazole 300 p.p.m. than 150 p.p.m. and with solutions containing 5-aminouracil 125 p.p.m. than 31 p.p.m. The percentage

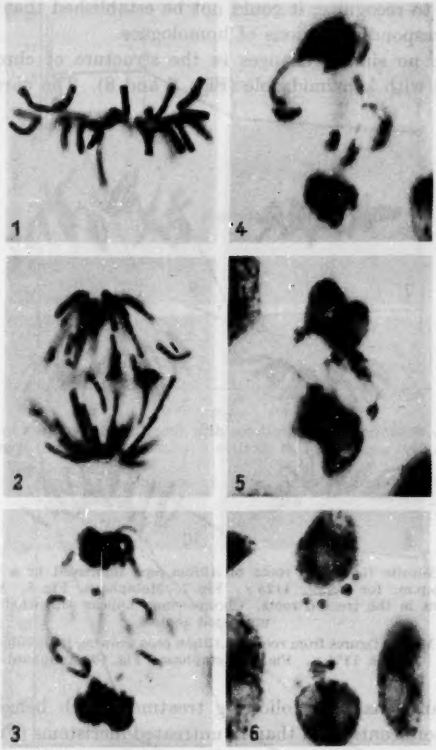
Table 1. *Effects of 24 hrs. treatment with various concentrations of benzimidazole and 5-aminouracil.*

	P.p.m.	% of nuclei in mitosis	% of anaphases with Feulgen-negative regions	% of newly formed cells with indisputable micronuclei
Control	—	9	0	0
Benzimidazole . .	150	7	0	0
	300	1	0	0
	600	0	0	0
Control	—	8	0	0
5-Aminouracil . .	31	5	26	0
	63	5	88	1
	125	2	68	0
	250	1	82	0
	500	2	100	1

of dividing nuclei is generally correlated with the concentration of the analogues. The higher the concentration, the fewer the divisions.

Five-aminouracil affects the structure of chromosomes (Figs. 1 and 2) with high frequency (second column, Table 1). The structural changes are especially noticeable at anaphase in roots treated for 24 hrs. After this duration of treatment many chromosome arms contain interstitial Feulgen-negative gaps; through each a thin strand evidently extends and this is occasionally visible. Frequently by late anaphase a Feulgen-negative gap may be so long that the distal trailing segment may be still at the equatorial region when the proximal portion of the chromosome is well on its way to the pole (Fig. 2). There is no evidence of stickiness in these cases though increase in length of the Feulgen-negative gap can easily be explained by difficulty in disjunction of sister chromatids. Although there are superficial resemblances, the trailing segments seem not to be free fragments, since their proximal ends are generally toward the poles and approach them (Figs. 3 and 4) during anaphase. Generally by the time sister nuclei are forming, no chromosome material remains near the equatorial plane of the spindle. Furthermore, the number of cells containing unquestionable micronuclei after 24 hrs. of treatment (third column, Table 1) shows little relationship to the number of cells containing anaphase groups of structurally modified chromosomes (second column, Table 1). In prophase and metaphase (Fig. 1) the Feulgen-negative regions are shorter and less frequent than at anaphase. At telophase a trailing distal segment may be so far from the polar group that it lies within a lobe of the newly constituted nucleus (Fig. 5). Frequently a number of what appear to be micronuclei lie close to the surfaces of sister nuclei adjacent to each other (Fig. 6). Apparently the lagging distal segments of the chromosomes are involved in the formation of the presumptive micronuclei as

well as the lobes of the nucleus. Occasionally a large nucleus at interphase is joined to smaller nuclei by delicate strands. More infrequent

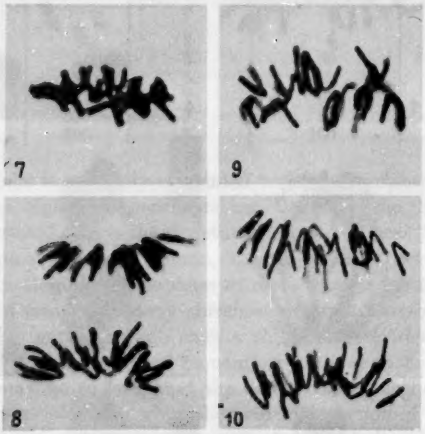


Figs. 1—6. The effects of 5-aminouracil on mitosis in root meristems of *Allium cepa*. Figs. 1 and 2, 125 p.p.m. for 24 hrs. Figs. 3—6, 50 p.p.m. for 24 hrs. Figs. 1, 3—6, 1125 ×. Fig. 2, 970 ×. Fig. 1. Metaphase. Fig. 2. Anaphase. The trailing distal portions generally appear to follow the proximal portions of the chromosomes to the poles and to be separated from them by a clear zone in which strands occasionally can be seen. Figs. 3—5. Figures illustrating the behavior of the distal portions of the chromosomes in division figures in which the proximal portions are already present at the poles. The figures are arranged in a sequence which suggests progressive approach of the distal portions of the chromosomes to the poles. Fig. 6. Sister nuclei with a group of what appear to be micronuclei lying near their adjacent faces. In many cases such nuclei may be more irregular in outline. An apparent micronucleus may be attached by a visible strand to a portion of the nuclear surface.

are unquestionable fragments and micronuclei, free of these attaching strands. None of these structures were observed in roots treated for 6 hrs.

and very few in those treated for 12 hrs. So far, corresponding Feulgen-negative regions have been observed to occur usually in both newly separated sister chromosomes. Since homologous chromosomes in onion are not easy to recognize, it could not be established that they always occur in corresponding regions of homologues.

There are no similar changes in the structure of chromosomes in roots treated with benzimidazole (Figs. 7 and 8). The chromosomes in



Figs. 7 and 8. Mitotic figures in roots of *Allium cepa* immersed in a solution of bezimidazole 500 p.p.m. for 24 hrs. 1125×. Fig. 7. Metaphase. Fig. 8. Anaphase. There are few divisions in the treated roots. Chromosomes appear somewhat shorter than in untreated roots.

Figs. 9 and 10. Mitotic figures from roots of *Allium cepa* growing in distilled water (control). Both 1125×. Fig. 9. Metaphase. Fig. 10. Anaphase.

metaphase and anaphase following treatment with benzimidazole are somewhat more contracted than in untreated meristems (Figs. 9 and 10).

The effect of duration of treatment with benzimidazole (300 p.p.m.) and 5-aminouracil (100 p.p.m.) on mitotic activity is shown in Fig. 11. Each point represents the average of the number of mitoses in five roots from five different bulbs. Soon after the beginning of treatment the number of nuclear divisions in roots treated with benzimidazole declines rapidly. The number of nuclear divisions in roots treated with 5-aminouracil, however, does not decline sharply for 6 to 12 hrs.

As a result of preliminary studies of antagonism between adenine sulfate and benzimidazole, a mixture of benzimidazole (300 p.p.m.) and adenine sulfate (800 p.p.m.) was chosen as a suitable ratio of analogue to metabolite for study. The effects of this mixture on the number of

nuclear divisions are shown in Fig. 12. Each point in Fig. 12 represents the average of the percentages of nuclei in division in five roots from

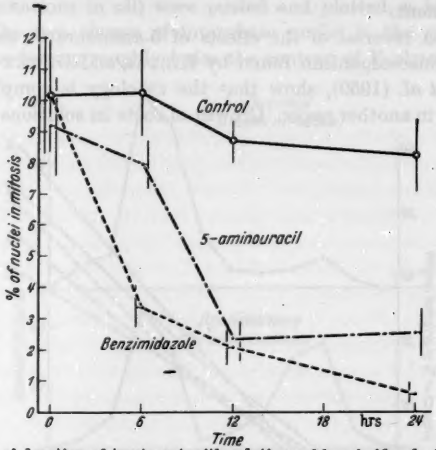


Fig. 11. Effect of duration of treatment with solutions of benzimidazole (300 p.p.m.) and 5-aminouracil (100 p.p.m.) on mitotic activity in *Allium cepa* root meristems.

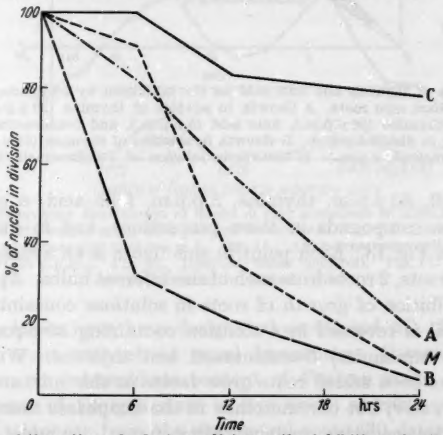


Fig. 12. Antagonistic action of adenine sulfate on the inhibition of mitotic activity in *Allium cepa* root meristems produced by benzimidazole. A. represents mitotic activity in roots immersed in solutions of adenine sulfate (800 p.p.m.); B, benzimidazole (300 p.p.m.); M, mixture (benzimidazole 300 p.p.m. and adenine sulfate 800 p.p.m.); C, distilled water.

five different bulbs. Adenine sulfate only temporarily reverses the block to mitosis caused by benzimidazole; it delays the decline in number

of mitoses for at least 6 hrs. Adenine sulfate itself reduces the number of nuclear divisions to some extent. This reduction is greater with longer treatment.

Studies on reversal of the effects of 5-aminouracil, similar to the reversals in microorganisms found by KIDDER and DEWEY (1949a) and HITCHINGS *et al.* (1950), show that the cytology is complex and will be discussed in another paper. Growth of roots in solutions of 50 p.p.m.

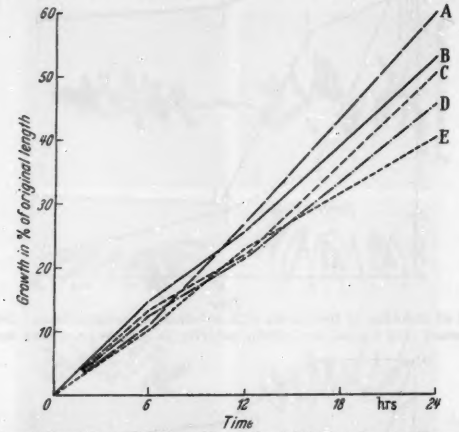


Fig. 13. Effects of thymine and folic acid on the inhibition by 5-aminouracil on growth in length of *Allium cepa* roots. A Growth in solution of thymine (50 p.p.m.). B Growth in solution of thymine (50 p.p.m.), folic acid (5 p.p.m.), and 5-aminouracil (50 p.p.m.). C Growth in distilled water. D Growth in solution of thymine (50 p.p.m.) and 5-aminouracil (50 p.p.m.). E Growth in solution of 5-aminouracil (50 p.p.m.).

5-aminouracil, 50 p.p.m. thymine, 5 p.p.m. folic acid, a solution containing these compounds in these proportions, and in distilled water are shown in Fig. 13. Each point in this figure is an average of growth made by 12 roots, 2 roots from each of six different bulbs. Approximately half the inhibition of growth of roots in solutions containing 50 p.p.m. 5-aminouracil is reversed in a solution containing 50 p.p.m. each (approximately equimolar) 5-aminouracil and thymine. When 5 p.p.m. folic acid has been added roots grow faster in this mixture than those in distilled water, but chromosomes in the anaphases therein still contained Feulgen-negative regions. Fifty p.p.m. thymine alone speed growth; 5 p.p.m. folic acid (an amount probably exceeding requirements) alone inhibits it more effectively then 5-aminouracil.

The effects of the two analogues on DNA synthesis were determined indirectly by measuring the content of Feulgen stain in nuclei in both treated and untreated root meristems. The content of Feulgen stain in

random nuclei in untreated root meristems falls into two classes (Fig. 16). When data from 30 nuclei from each of six roots from different bulbs (180 determinations in all) were pooled and plotted, a bimodal curve resulted. The two classes of interphase nuclei in the root meristem differ by one having roughly twice the content of Feulgen stain of the

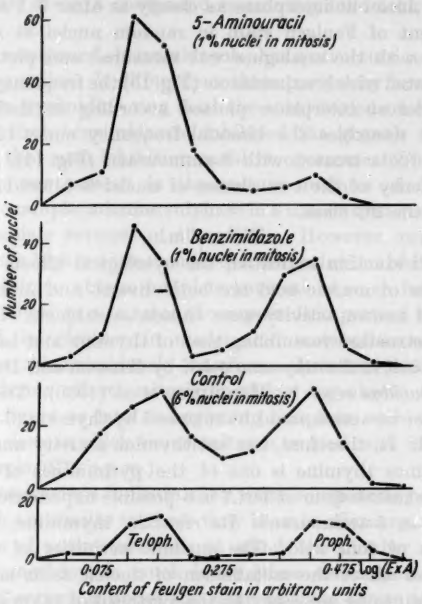


Fig. 14.

Fig. 15.

Fig. 16.

Fig. 17.

Figs. 14—17. Frequency distribution of nuclei in root meristems of *Allium cepa* according to their content of Feulgen stain after various treatments. Fig. 14. After treatment with a solution of 5-aminouracil (100 p.p.m.). Fig. 15. After treatment with a solution of benzimidazole (300 p.p.m.). Fig. 16. Distilled water (control). Fig. 17. Telophase and prophase nuclei in control roots.

other. The classes have been designated as 4C and 2C. Nuclei of the two classes occur with about equal frequencies. Comparatively few nuclei possess an intermediate content of Feulgen stain.

The content of Feulgen stain in five nuclei at early prophase and five at late telophase from the six control roots (30 determinations of each) are presented in Fig. 17. Nuclei at prophase have about the same content of Feulgen stain as 4C nuclei at interphase, and nuclei at telophase have the same amount as the 2C interphase nuclei. The average of the percentages of nuclei in division in the meristems of the six roots from a treatment is presented above the graphs (Figs. 14—16) illus-

trating each treatment. This average, as well as those for roots treated with either of the two antimetabolites, is somewhat lower than those obtained after similar treatments in an earlier experiment (see Fig. 11). A factor which contributed to this difference is that after formalin fixation nuclei at early prophase and late telophase could not be distinguished from nuclei at interphase as clearly as after 3:1 alcohol-acetic.

The content of Feulgen stain in random nuclei at interphase of roots treated with the analogues was measured and plotted. In root meristems treated with benzimidazole (Fig. 15) the frequency distribution curve of nuclei at interphase plotted according to their content of Feulgen stain resembles the bimodal frequency curve of the control (Fig. 16). In roots treated with 5-aminouracil (Fig. 14), however, the relative frequency of the two classes of nuclei is altered; most of the nuclei are in the 2C class.

Discussion.

As the introduction indicated, the cytological effects of analogues of components of nucleic acid are both diverse and interesting. Two compounds of known activity were chosen as subjects. One, 5-aminouracil, has a structure resembling that of thymine and has been found to inhibit growth in *Tetrahymena geleii* by KIDDER and DEWEY (1949a) and in *Lactobacillus casei* by HITCHINGS *et al.* (1950). The latter found that in *L. casei* its action could be reversed by thymine (5-methyluracil) and folic acid. It, therefore, has antithymine activity and antagonizes folic acid. Since thymine is one of the pyrimidines of DNA, interference in the metabolism of DNA is a possible explanation of one role of its analogue, 5-aminouracil. Its riboside, thymidine, is involved in the functions of folic acid. The antifolic activities of 5-aminouracil would likewise affect the metabolism of nucleic acids since folic acid has been shown to be necessary to the synthesis of thymine and purines or their equivalents (STOKES 1944; LAMPEN and JONES 1947; SHIVE, EAKIN, HARDING, RAVEL, and SUTHERLAND 1948; ROGERS and SHIVE 1948; and WOOLLEY and PRINGLE 1950) and has been shown to be involved directly in nucleic acid synthesis (PRUSOFF, TEPLY, and KING 1948; and SKIPPER, MITCHELL and BENNETT 1950). Interference with thymine metabolism and folic acid activity, then, would have far reaching consequences on the synthesis of nucleic acids. Both thymine and folic acid presumably are involved in nucleic acid metabolism in onion.

The cytological effects of compounds known to be antagonists of thymine have not been studied to any great extent. The antifolics, however, are known to affect mitosis. No anaphases and telophases were present in material HIMES and LEUCHTENBERGER (1950) treated with solutions of an analogue of folic acid, aminopterin (4 amino pteroylglutamic acid). HUGHES (1950) pointed out that aminopterin destroyed the spindle in dividing fibroblasts in chick tissue cultures. The chromo-

somes did not move to the poles from the metaphase plate. JACOBSON (1952) also ascribed the absence of late stages in mitosis after such treatment to the failure of chromatids to separate and move from the equatorial region of the spindle to the poles. While folic acid is impotent in overcoming this affect, folinic acid (5 formyl 5, 6, 7, 8 tetrahydro pteroylglutamic acid), a compound resembling folic acid, brings about a reversal.

The other compound, benzimidazole, has a structure resembling that of adenine or guanine. It differs from these purines by having a benzene in place of the pyrimidine ring (and the substitution on it). WOOLLEY (1944) found that benzimidazole inhibited the growth of yeasts and bacteria, and that such inhibition could be overcome. When 600 p.p.m. benzimidazole was present in the culture medium of *Saccharomyces cerevisiae*, for example, adenine sulphate in a concentration of 1000 p.p.m. produced complete reversal of the effects. However, reversal usually is not possible in higher animals injected with solutions of benzimidazole, nor is it possible to obtain complete reversal in some bacteria. Since adenine is involved in a number of compounds important in the physiology of a cell, interference in nuclei acid metabolism cannot be the only area where benzimidazole interferes. HUGHES (1952) has found that benzimidazole affects mitosis in higher organisms. Chromosomes in a division figure as late as beginning anaphase were reconstructed as a single nucleus in chick tissue cultures treated with 8 mM. solution of benzimidazole (974 p.p.m.).

Several observations indicate that these compounds have activities in onion which are similar to those they have in microorganisms: 1. The concentrations of antimetabolites, at which the number of mitoses in the immersed meristem is much lower (than concentrations following it in a dilution series), is about the same as those concentrations found critical for inhibition of growth of microorganisms. Naturally, to equate inhibition of cell multiplication or lactic acid production in microorganisms with inhibition of mitoses in an onion root tip may be oversimplification. 2. Furthermore, microorganisms and onion respond similarly to attempts at producing reversals. Overcoming half the inhibition of growth of onion roots in a solution containing 50 p.p.m. 5-aminouracil by the additions of 50 p.p.m. thymine or entirely overcoming it by adding 5 p.p.m. folic acid to this mixture is evidence that 5-aminouracil is an antithymine and antagonizes folic acid activities in onion as well as *Lactobacillus casei*. This concentration of 5-aminouracil is higher than the concentration at which HITCHINGS *et al.* (1950) obtained complete reversal in *L. casei*. While reversal of inhibition of growth in onion is complete for 24 hrs., chromosomes with Feulgen-negative gaps are still present at anaphase. Perhaps recovery from this phase of the effects of 5-aminouracil need not be expected with any concentration

of thymine since it may not be the precursor of thymine in nucleic acid. If the effect of 5-aminouracil on chromosomes results from its antagonizing the activity of a compound closely related to folic acid such as folinic acid, addition of even excessive amounts of folic acid to the solution bathing the roots could not overcome this effect.

Preventing a sharp decline in number of mitoses for six hours in roots of onions immersed in solutions containing 300 p.p.m. benzimidazole by the addition of 800 p.p.m. adenine sulphate suggests that benzimidazole has anti-adenine activity in onion. Reversal of the effects on mitoses in onions at concentration of benzimidazole where its effects were completely reversed in yeasts, was not successful. Apparently onion, like the higher animals does not respond to attempts at producing reversals.

In onion the effects of these analogues on nucleic acid metabolism can be analyzed in some detail by photometric methods. In untreated meristems there are approximately equal numbers of nuclei with a smaller content of Feulgen stain (2C) and with twice (4C) this amount. Nuclei in late telophase are 2C; nuclei in early prophase are 4C. The 2C and 4C values are equivalents of the content of DNA in a nucleus. Evidently, doubling the DNA occurs during interphase and a nucleus comes to have the 4C DNA equivalent before it divides. Nuclei in telophase have a 2C DNA equivalent since halving has, of course, occured during nuclear division. These conclusions are in agreement with those of SWIFT (1950a and b) and NELSON (1951).

Meristems treated with 5-aminouracil have a larger proportion of 2C nuclei in interphase than untreated meristems have. Two observations may help explain how this comes about. First, as far as can be detected, the spindle is not disturbed; nuclear divisions which are incomplete at the beginning of treatment go to completion. Second, there is no pronounced decrease in the number of cells undergoing mitosis until about 6 hrs. after the beginning of treatment. Evidently, the nuclei which have previously doubled their amount of DNA to 4C in readiness for division, can still divide even though the meristem is being treated with 5-aminouracil. Their daughter nuclei are 2C. Eventually the majority of 4C nuclei which were present at the beginning of treatment are replaced by 2C daughter nuclei. But, apparently, transition from the 2C to 4C level is prevented. To say the transition does not occur is to say that DNA does not accumulate in the nucleus and to suggest that its synthesis is blocked. This shift from the condition where approximately equal numbers of 2C and 4C nuclei are present in untreated meristems to one where there is a majority of 2C nuclei, along with the drop in number of mitotic divisions, provides still more evidence that: 1. doubling of the DNA content of a nucleus occurs during the interphase, and 2. that there is a relationship between the DNA content of a nucleus in the root meristem and its ability to divide.

It was found that 2C and 4C nuclei occur in approximately equal numbers in root meristems immersed for 24 hrs. in a solution containing 300 p.p.m. benzimidazole. These treated meristems resemble untreated ones in this respect but the resemblance ends there. The percentage of nuclei undergoing mitosis in the treated meristem is much lower than in untreated ones. Since the number of 4C nuclei has not become disproportionate the amount of DNA synthesized must be lower. Hence both the mitotic cycle and DNA synthesis have been affected. Halving of the 4C amount of DNA is blocked because nuclei are no longer dividing. Doubling of the 2C amount of DNA is also evidently blocked. If these two blocks were about equally efficient and became effective at the same time, the relative numbers of 2C and 4C nuclei would remain unaffected by the treatment.

The block which prevents the shift from the 2C to the 4C condition of the nucleus appears to be a direct result of interference in DNA synthesis. Benzimidazole and 5-aminouracil both act in this way. The structural change in the chromosomes of roots treated with 5-aminouracil, and the apparent block to prophase formation in roots treated with benzimidazole are effects which are much more difficult to interpret for they are characteristic of the particular compound used. For the moment these differencs must be attributed to intrinsic factors of the compounds themselves.

A gross structural difference between the two chemicals is that one (benzimidazole) is the analogue of the purines of nucleic acid and the other (5-aminouracil) is an analogue of thymine. The reversals of their effects obtained by the appropriate metabolites indicate that their analogues do interfere in adenine and thymine activities respectively. The localized erosion of chromosomes in meristem treatment with 5-aminouracil, however, cannot be ascribed to the absence of only nucleotides containing thymine (though their quantity may be reduced), because folic acid metabolism is also involved. In this event, synthesis of nucleic acid might be more generally affected. Furthermore pyrimidines are not freed from DNA by the type of hydrolysis used in making the Feulgen preparations for this study, although purines are (DI STEFANO 1948). In this light the Feulgen technique can be taken as a test for purines and the interpretation of the nature of the Feulgen-negative gaps is problematic. They at least represent regions where purines are infrequent and after proper reversal may come to be understood as regions where interference in thymine and folic acid or folinic acid activity has a disruptive effect on the structure of a chromosome.

Five-aminouracil has other structural features which may influence its mode of action. For example, the amino substitution present on the pyrimidine ring may, as has been suggested for amino groups in general, disturb hydrogen bonding, and, consequently, polymerization of nucleic acids. Such interference might be expected to be general rather than

localized along the chromosome length. This is not to deny that 5-aminouracil has a general effect. Indeed there are indications that chromosomes in the cells of a meristem treated with 5-aminouracil are both somewhat longer and broader than in untreated cells. This possibility, however, needs further evaluation. An amino substitution on the ring is not necessary for a pyrimidine base to fragment chromosomes. DEYSSON (1952) reported that a single chromosome in the somatic chromosome complement of *Allium cepa* is frequently broken in mitosis in roots treated with 0.25% solutions of uracil for 6 hrs.

The failure of benzimidazole to affect the structure of chromosomes is not characteristic of all analogues of the purines in nucleic acids. KIHLMAN (1952) has investigated the effects of many compounds of this nature. Some produce erosion and fragmentation of chromosomes and bring about a decrease in the percentage of nuclei in division. He ascribed some of their activities to the solubilizing power of purines. Benzimidazole differs from these purines in that it has a benzene ring in place of the pyrimidine ring. It is improbable that this difference in itself is responsible for this difference in activity. Perhaps benzimidazole would break and erode chromosomes either in other subjects or under other experimental conditions. Inhibiting new mitoses prevents halving of the amount of DNA within a nucleus and also disclosure of any effects on chromosomes that benzimidazole may have. In this way inhibition of mitosis is a protective device against whatever disruptive effects mitoses may have on damaged chromosomes. The nature of this block to halving the amount of DNA is not understood. If reconstitutions, similar to those HUGHES (1952) saw in chick tissue culture, follow treatment with 300 p.p.m. benzimidazole in onion, reduction in the number of nuclei with the 4C DNA equivalent would be prevented. Photometric measurements of DNA content would not determine, of course, whether the nucleus was 4n and had a 4C DNA equivalent after reconstitution or was 2n and had a doubled (4C) DNA equivalent ready to enter prophase.

Summary.

The effects of solutions of benzimidazole and 5-aminouracil on *Allium cepa* root meristems immersed in them have been studied cytologically. Benzimidazole and 5-aminouracil, analogues of purines and pyrimidines respectively, reduce the number of nuclear divisions being initiated. A marked decline in frequency of cells in mitosis occurs at the beginning of treatment with benzimidazole (300 p.p.m.) but not until 6 or more hours later with 5-aminouracil (125 p.p.m.).

After treatment with 5-aminouracil, small interstitial portions of chromosomes are Feulgen-negative and become extended during anaphase into thin strands. The distal portion of these chromosomes may lag during anaphase and contribute to malformations of the surface of

the sister nuclei and to micronuclei-like bodies lying along their adjacent surfaces. However, stickiness resulting in chromosome bridges was not observed. After treatments with benzimidazole, chromosomes are more contracted at metaphase and anaphase than in the control. At the concentrations used, neither component was observed to affect the spindle mechanism of the divisions in progress at the beginning of treatment nor of the divisions which escape inhibition.

Reversal of these effects by the introduction of metabolites which these analogues resemble structurally, was investigated. Adenine sulphate (800 p.p.m.) acts antagonistically to benzimidazole (300 p.p.m.) by delaying the marked decline in frequency of mitoses by more than 6 hrs. By 12 hrs. the frequency of cells in mitoses was very low; thus, reversal is not permanent. Half the inhibition of growth caused by 5-aminouracil (50 p.p.m.) is reversed by thymine (50 p.p.m.) for the 24 hrs. of the test period. When 5 p.p.m. folic acid was added to this mixture, root growth exceeded that of the controls. Feulgen-negative gaps, however, are still present in chromosomes at anaphase.

Estimations of Feulgen content of nuclei to determine the effect of these treatments on DNA synthesis were made by means of a microphotometer. In control roots there are two classes of nuclei at interphase according to the amount of DNA per nucleus. The amount doubles during interphase before the next division of the nuclei in the root meristem. In roots treated with 5-aminouracil this doubling is inhibited, so that interphase nuclei with the lower amount of DNA accumulate. In roots treated with benzimidazole the relative frequencies of the two classes of nuclei is unaltered; however, mitotic activity is inhibited. This compound apparently affects not only processes concerned with doubling the amount of DNA per nucleus but also processes necessary to the division itself. There was, however, no unquestionable evidence of a nucleus being reconstituted after it had started a division.

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THE DESOXYRIBOSENUCLEIC ACID (DNA) CONTENT IN SPERMATOZOA OF FERTILE AND INFERTILE HUMAN MALES*.

By

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With 11 figures in the text.

(Eingegangen am 1. Juli 1953.)

I. Introduction.

The analysis of the spermatozoa in the seminal fluid constitutes one of the fundamental criteria in studying the complex problem of infertility in the human male. While it is generally recognized that volume and viscosity of semen, as well as counts, motility and morphology of the spermatozoa give basic information in the appraisal of fertility, there is no agreement concerning the decisiveness of any one of these factors in determining whether the sperms of a male are capable of normal fertilization. Some workers (MEAKER 1934, MACOMBER and SAUNDERS 1929), attribute great importance to the concentration of the sperms, but others, as for example MOENCH (1936) minimize this factor and correlate infertility with the occurrence of abnormal morphology of the sperms. HOTCHKISS in his excellent book "Fertility in Men" (1944) concludes that it is not possible to use any one single factor (such as sperm count, morphology, or motility) to ascertain fertility, but that it is necessary to correlate all of the known data on the semen with the findings obtained by the physical examination of the males to justify a division into fertile and sterile categories.

In the present study we are concerned with the sperms themselves rather than with other factors which may also play a role in bringing about sterility. The difficulties in gauging spermatozoa for their fertilizing ability on the basis of the known criteria, clearly indicate a lack in our knowledge of the detailed structure of spermatozoa and suggest the presence of still unexplored elements which may play a decisive role in the characterization of a sperm as fertile or sterile. While in

* This study was supported by two grants from the Brush Foundation of Cleveland, Ohio.

recent years, investigations dealing with the cytology (Baylor, Nalbandov and Clark 1943; Bretschneider and Van Iterson 1947; Hodge 1949; Randall and Friedlander 1950; Reed and Reed 1948; Seymour and Benmosche 1941; Schnall 1952), — chemical composition (Clermont and Leblond 1950; Elftmann 1952; Hechter and Hadidian 1947; Leuchtenberger, Vendrely and Vendrely 1951; Leuchtenberger, Leuchtenberger, Vendrely and Vendrely 1952; Long and Engle 1952; Riisfeldt 1949; Wislocki, 1950), — and metabolic activity of mammalian spermatozoa (Henle and Zittle 1942; Lardy, Ghosh and Plaut 1949; MacLeod 1946; MacLeod and Summerson 1946; MacLeod 1951), have contributed pertinent data, the obstacles encountered in correlating these findings with fertility demonstrate the need for further intensive work on the spermatozoa.

The present report which deals with the amount of desoxyribosenucleic acid (DNA) in spermatozoa of human fertile and infertile males is an attempt to relate one of the most important chromosomal components to the fertilizing ability of the sperm. In spite of the increasing recognition of the DNA as a significant nuclear component of all animal and plant cells, and the vast amount of research done on DNA in the last decade (for review paper, see Leuchtenberger 1953) there is actually very little known in regard to the DNA of the human sperm. This is probably due to the fact that until very recently there were no chemical methods available for the extraction of DNA from human spermatozoa. In 1950, Zamenhof, Shettles and Chargaff (1950) isolated for the first time a highly polymerized DNA from human sperms, but did not present any data as to the anmouts of DNA in such sperms. Thus to our knowledge the present study is the first attempt to evaluate quantitatively the content of DNA in human sperms by cytochemical methods. The validity of the quantitative cytochemical microspectrophotometric methods in determining the DNA in individual mammalian sperms has been established in previous studies by Leuchtenberger, Leuchtenberger, Vendrely and Vendrely (1952). These workers found that the amounts of DNA in individual bull sperms as determined by ultraviolet and Feulgen microspectrophotometry agreed closely with the results of DNA estimation done on the same bull sperms by biochemical analysis.

II. Material and Methods.

In order to carry out the investigations to be described, seminal fluids of both fertile and infertile males were secured. From the Maternity Clinic of the University Hospitals in Cleveland, Ohio, samples were obtained from 21 men each of whom had fathered from 1—3 children and thus represented cases known to be fertile. From the Infertility Clinic of the Maternal Health Association of Cleve-

land, samples were obtained from the male partners of 18 childless couples. These represented cases of suspected male infertility, although it was not definitely known which partner was responsible. The samples were procured after an interval of at least two days following the previous ejaculation. This was done to prevent depletion of semen by too frequent ejaculation. The count, motility and morphology of the spermatozoa were determined on samples not older than 2—3 hours by the standard methods of which a brief description is given here (HOTCHKISS 1944).

1. Count. The count is an estimation of the number of sperms per cubic centimeter of seminal fluid and is made by the pipette dilution method used for the counting of blood cells. 0.5 cumm. of the seminal fluid is drawn into a white blood cell pipette and diluted 1:20 with a solution of 1% phenol in saturated sodium carbonate, shaken, and one-half the volume discarded. One drop is placed in a Neubauer counting chamber and 5 blocks of 16 squares are counted three separate times. The average multiplied by one million is used as the final result for the count.

2. Motility. The estimation of motility is based on the percentage of motile sperms and on the degree of their activity. When the sample is first received, a wet drop is placed on a slide and the motility is recorded immediately. The total number of sperms present does not affect the grading; it is used only as a basis for computing the percentage of motile sperms in the sample. Thus, for example, one sample may contain numerous sperms, but of these only 5% may show motility, whereas in a second sample there may be only a few sperms present but of these 95% may show motility. Further to characterize the motility of the sperms, the following grading established by HOTCHKISS (1944) was used:

1. — — — — Sluggish activity
2. — — — — Poor to fair activity
3. — — — — Good activity
4. — — — — Excellent activity.

The final classification thus expresses both degree and percentage of motility; a grading of $50\%_2$, for example, indicates that although half the sperm were motile, their activity was only fair.

3. Morphology. With the same wet drop used in determining the motility, a smear is made and allowed to dry. The dried smear is fixed in 10% formalin for 1 min, rinsed in water, staind with Mayer's hematoxylin for one and one-half minutes and rinsed again in water. The stained smear is allowed to dry, and the morphological characteristics are then observed under oil immersion. The sperms are graded into normal oval forms and abnormal forms; the latter include large, small, tapering, round, duplicate and amorphous types.

4. Estimation of DNA in individual human sperm. For the determination of the amounts of DNA, the seminal fluids were centrifuged and from the resulting sediment, smears were made. The smears were not allowed to dry, but were placed immediately into Carnoy fixing fluid (1:3 of glacial acetic acid and 95% alcohol) for 5—10 min, washed in three changes of 95% alcohol, brought down to water through lower alcohols and subjected to the Feulgen reaction according to the standard procedure (DISTEFANO 1948; STOWELL 1945). In addition, paraffin blocks were made from the same centrifuged sediment. Sections were cut at 3 microns and stained by the same Feulgen method. For the estimation of the amouts of DNA in the Feulgen stained slides, microspectrophotometric measurements on individual sperms were made as previously described. (LEUCHTENBERGER 1950; SCHRADER and LEUCHTENBERGER 1950). It should be emphasized that only morphologically normal sperms with a homogeneous distribution of the DNA were selected for the measurements.

5. Clinical study of infertile couples. The clinical studies of the infertile couples were performed according to the standards established by the American Society for the Study of Sterility (1951) except for the fact that endometrial biopsies were not done. Ovulation was assumed to occur if the basal body temperature curves were normal. Additional procedures such as salpingograms, excretion of hormones, or psychiatric studies were used when indicated. A final evaluation of all the findings was made and is included in Table III under Clinical Findings. The cases were selected as representative patients from the last 160 couples of our series, which, at the time of writing, numbers 425. Selection was made by the senior author. Until her observations were completed, she had no knowledge of the clinical status of the patients except for the fact that they had been admitted to the clinic for infertility studies.

Table 1. *Amount of DNA (microspectrophotometry) and microscopical findings in spermatozoa of human males with proven fertility.*

Case No.	Sperm count	Sperm motility	Morphology		Mean amount of DNA per sperm
			normal	abnormal	
1	17 M	$60\%_2$	70%	30%	$1.29 \pm .01$
2	24 M	$90\%_3$	87%	13%	$1.24 \pm .01$
3	28 M	$70\%_4$	73%	27%	$1.21 \pm .03$
4	30 M	$90\%_4$	73%	27%	$1.23 \pm .01$
5	33 M	$80\%_4$			$1.20 \pm .02$
6	45 M	$80\%_4$	85%	15%	$1.22 \pm .02$
7	46 M	$50\%_3$	79%	21%	$1.24 \pm .02$
8	48 M	$60\%_4$			$1.22 \pm .02$
9	54 M	$80\%_3$	70%	30%	$1.23 \pm .02$
10	69 M	$60\%_2$	75%	25%	$1.30 \pm .01$
11	84 M	$40\%_2$	55%	45%	$1.18 \pm .02$
12	133 M	$70\%_4$	62%	38%	$1.23 \pm .01$
13	176 M	$90\%_4$			$1.18 \pm .01$
14	127 M	$70\%_4$	62%	38%	$1.19 \pm .02$
15	88 M	$80\%_3$	71%	29%	$1.19 \pm .01$
16	67 M	$80\%_3$	75%	25%	$1.20 \pm .02$
17	6 M	$60\%_3$	60%	40%	$1.25 \pm .02$
18	186 M	$95\%_4$	74%	26%	$1.21 \pm .02$
19	83 M	$85\%_3$	62%	38%	$1.25 \pm .02$
20	111 M	$80\%_3$	83%	17%	$1.20 \pm .02$
21	20 M	$80\%_2$	59%	41%	$1.24 \pm .03$

III. Results.

A. DNA values in spermatozoa of males with proven fertility.

The results of the DNA measurements in individual spermatozoa in relation to counts, motility and morphology of spermatozoa of 21 males with proven fertility are presented in Table 1.

It is evident from this table that the numbers, motility and morphology of spermatozoa vary considerably within the group of these 21 fertile males. The counts exhibit a range from 6 million to 186 million in different individuals; the motility shows values as low as 40% and as high as 95% and the degree of motility varies from 2 to 4; and finally the occurrence of abnormal spermatozoa may be 13% for one individual and 45% for another.

In contrast to the variations in counts, motility, and morphology of spermatozoa observed in these 21 males, the amounts of DNA in their spermatozoa show a remarkably narrow range of values. The mean amounts of DNA in these 21 cases vary between 1.18—1.30 or, in other words, the most extreme variation which occurred between 2 individuals of the total group of 21 is 9%. Furthermore, as can be

seen from Table 2, the mean amounts of DNA in spermatozoa of semen samples of the same individuals taken at different periods show very little variation.

In accordance with this slight variation of the mean DNA values among different fertile individuals, the amounts of DNA in *individual spermatozoa* of the same males and within the total group also do not deviate markedly from each other. In Fig. 1, the DNA values for all the 563 spermatozoa, are recorded.

From these data, it can be seen that 493 (88%) of the 563 spermatozoa have DNA values between 1.15—1.35, that is, a range in which the highest DNA value differs by only 15% from the lowest one. Moreover none of the DNA values differs by more than 15% from the computed *mean* DNA value of 1.22 $\pm$ 0.005. It should also be noted that, with but two exceptions, all the spermatozoa have a DNA value above 1.05; the significance of this finding will be apparent after the consideration of DNA values in sperms from infertile males.

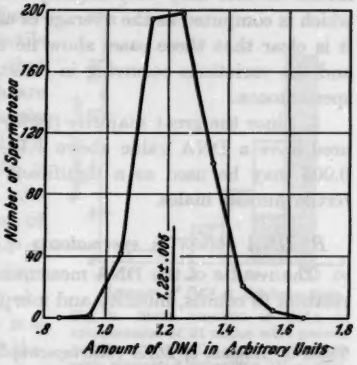

Fig. 1. Amount of DNA (microspectrophotometry of Feulgen reaction) in individual spermatozoa of 21 human males with proven fertility. Number of spermatozoa measured N = 563, mean amount of DNA represented by vertical line.

Table 2. *Amount of DNA in spermatozoa of Cases 1 to 8, from samples taken at later dates than Table 1.*

Case No.	Date specimen	Count	Motility	Morphology		Mean amount of DNA
				normal	abnormal	
1	12. 10. 52	127 M	$70\%_4$	62%	38%	1.13 $\pm$ .016
	1. 7. 53	48 M	$80\%_3$	68%	32%	1.25 $\pm$ .016
2	12. 10. 53	170 M	$95\%_4$	76%	24%	1.15 $\pm$ .015
	1. 5. 52	88 M	$80\%_3$	71%	29%	1.23 $\pm$ .014
3	12. 29. 52	67 M	$80\%_3$	75%	25%	1.14 $\pm$ .012
	1. 5. 53	123 M	$90\%_4$	75%	25%	1.26 $\pm$ .018
4	12. 15. 52	5 M	$50\%_3$	50%	50%	1.27 $\pm$ .001
	1. 12. 53	6 M	$60\%_3$	60%	40%	1.21 $\pm$ .019
	2. 11. 53	8 M	$70\%_3$	60%	40%	1.28 $\pm$ .023
5	1. 14. 53	186 M	$95\%_4$	74%	26%	1.20 $\pm$ .016
	1. 27. 53	160 M	$95\%_4$	70%	30%	1.23 $\pm$ .022
6	1. 28. 53	83 M	$85\%_3$	62%	38%	1.28 $\pm$ .025
	2. 12. 53	84 M	$60\%_2$	65%	35%	1.22 $\pm$ .019
7	12. 19. 52	111 M	$80\%_3$	83%	17%	1.20 $\pm$ .023
8	12. 29. 52	20 M	$80\%_2$	59%	41%	1.24 $\pm$ .030

On the basis of the data presented in Table 1 and Fig. 1, the following conclusions may be drawn:

1. The number, motility, and morphology of the spermatozoa of 21 males with proven fertility (fathers of 1—3 children) show considerable variation and range among different individuals.

2. The mean amount of DNA in the sperm of any one individual is very close to that of any other male in this group. The individual means deviate only very slightly from the mean DNA value of 1.22 which is computed as the average of all individual means. Consequently it is clear that these cases show no correlation between DNA content and the variations occurring in count, motility and morphology of the spermatozoa.

3. Since the great majority (92%) of all the 563 spermatozoa measured have a DNA value above 1.15, the mean DNA value of 1.22 ± 0.005 may be used as a significant criterion for the spermatozoa of fertile human males.

B. DNA values in spermatozoa of males with suspected infertility.

The results of the DNA measurements in individual spermatozoa in relation to counts, motility and morphology of spermatozoa of 18 males

Table 3. *Amount of DNA (microspectrophotometry) and microscopical findings in spermatozoa of human males with proven fertility and suspected infertility.*

Proven fertility				Suspected infertility			
Case No.	Sperm count	Sperm motility	Mean amount of DNA per sperm	Mean amount of DNA per sperm	Sperm count	Sperm motility	Case No.
1	17 M	$60\%_2$	$1.29 \pm .01$	$1.17 \pm .02$	7 M	$30\%_2$	1
2	24 M	$90\%_3$	$1.24 \pm .01$	$.97 \pm .01$	8 M	$70\%_3$	2
3	28 M	$70\%_4$	$1.21 \pm .03$	$1.08 \pm .03$	12 M	$50\%_1$	3
4	30 M	$90\%_4$	$1.23 \pm .01$	$1.10 \pm .02$	15 M	$70\%_4$	4
5	33 M	$80\%_4$	$1.20 \pm .02$	$.91 \pm .01$	12 M	$70\%_3$	5
6	45 M	$80\%_4$	$1.22 \pm .02$	$1.15 \pm .02$	18 M	$10\%_1$	6
7	46 M	$50\%_3$	$1.24 \pm .02$	$1.18 \pm .02$	21 M	$50\%_4$	7
8	48 M	$60\%_4$	$1.22 \pm .02$	$.89 \pm .01$	24 M	$80\%_4$	8
9	54 M	$80\%_3$	$1.23 \pm .02$	$1.08 \pm .02$	56 M	$80\%_4$	9
10	69 M	$60\%_2$	$1.30 \pm .01$	$1.12 \pm .01$	58 M	$90\%_4$	10
11	84 M	$40\%_2$	$1.18 \pm .02$	$1.02 \pm .02$	72 M	$85\%_4$	11
12	133 M	$70\%_4$	$1.23 \pm .01$	$1.14 \pm .01$	78 M	$60\%_3$	12
13	176 M	$90\%_4$	$1.18 \pm .01$	$.76 \pm .01$	78 M	$80\%_4$	13
				$1.19 \pm .01$	114 M	$50\%_3$	14
				$1.00 \pm .01$	166 M	$90\%_4$	15
				$.88 \pm .01$	231 M	$70\%_3$	16
				$.98 \pm .02$	16 M	$50\%_2$	17
				$1.54 \pm .003$			
				$1.98 \pm .08$			
				$1.05 \pm .003$	27 M	$50\%_3$	18
				$1.60 \pm .04$			
				$1.87 \pm .04$			

with suspected infertility are presented in Table 3. For direct comparison the DNA data of 13 males with proven fertility, studied within the same period as the males with suspected infertility, are again presented.

It is evident from Table 3 that, as in fertile males, the numbers, the motility and morphology of the spermatozoa vary considerably also within this group of males with suspected infertility. But it is also apparent that the mean DNA values for these 18 cases show a much greater variation among themselves than did those of the fertile group. In addition, it can be seen that there are mean DNA values which are significantly lower and significantly higher than any one of the mean DNA values of the 21 fertile males[1]. Fig. 2 presents a comparison between the mean DNA values of spermatozoa of 21 males with proven fertility and of 18 males with suspected infertility. From the histogram, the following observations can be made: 1. There is a marked difference in the spread of the mean DNA values between the fertile and "suspected infertile" group. While practically all fertile males show a mean DNA value between 1.21—1.30 (only four had 1.18 or 1.19) thus presenting a uniform population in regard to the DNA content of spermatozoa, the heterogeneity of the group with "suspected infertility" is striking.

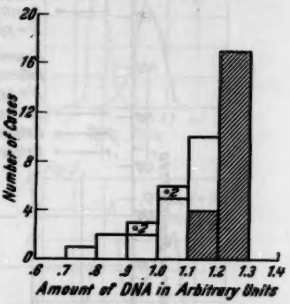

Fig. 2. Mean amount of DNA in spermatozoa of 21 males with proven fertility (N = 563), and of 18 males with suspected infertility (N = 586).
▨ cases with proven fertility;
□ cases with suspected infertility;
×2 cases with second and third means (1.60, 1.87, 1.54, 1.98) of DNA.

There are actually 3 categories within this group of "suspected infertile" males. As indicated in Fig. 2, the first category of six cases shows mean DNA values between 1.11—1.20, that is, amounts very close to the one found in the fertile males. The second category of 5 cases has mean values of 1.01—1.10 which for convenience might be called "borderline DNA values". The third category of 7 cases shows amounts of DNA between 0.71 and 1.10 with additional second and third peaks of 1.50 and 1.91 in two individual cases), and which are here classified as "abnormals" in regard to their mean DNA values.

[1] Since this manuscript was submitted for publication, studies made on samples taken at different intervals from the same individuals show fluctuations of the DNA content in this group. This finding is in contrast to the remarkable stability found in the repeat samples of the fertile group. A detailed report of this work will appear elsewhere.

In Fig. 3, the DNA values in *individual spermatozoa* of these 3 categories of the "suspected infertile group" and of the fertile group are presented.

In comparing the curve of the fertile group with the curve of category I of the "suspected infertility group" one is impressed by the similarity of the DNA content in the individual spermatozoa of both.

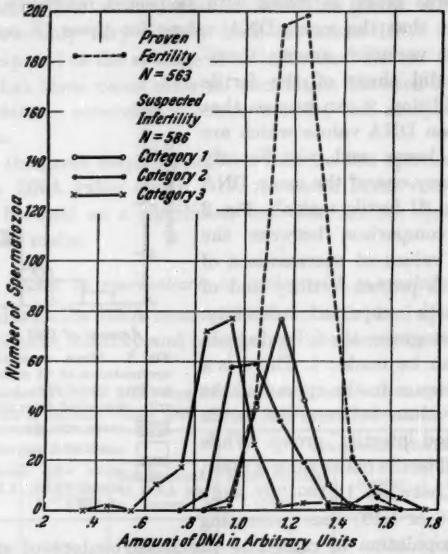

Fig. 3. Amount of DNA in individual human spermatozoa of 18 males with suspected infertility, separated into three categories (N = 586), in comparison with those of 21 males with proven fertility (N = 563).

In both instances, nearly 90% of the spermatozoa have DNA values which lie within 10% of the mean DNA content per sperm.

Examining the DNA data of the spermatozoa of category II, a slight but significant shift towards lower DNA values is seen, the mean amount of DNA being 1.06 as compared with 1.16 of category I and 1.23 of the group with proven fertility.

The occurrence of the lower DNA values in spermatozoa is especially pronounced in category III. 88% of 228 spermatozoa measured have DNA values significantly lower than those of the group with proven fertility and of category I. The difference between the DNA values of this category III and those of the fertile group or of category I is especially striking if one considers the fact that only two sperms of cate-

gory III have a DNA value at the DNA peak of the two others groups, and that none of the sperms of the two latter groups has a DNA value at the peak of category III; in other words there is practically no over-

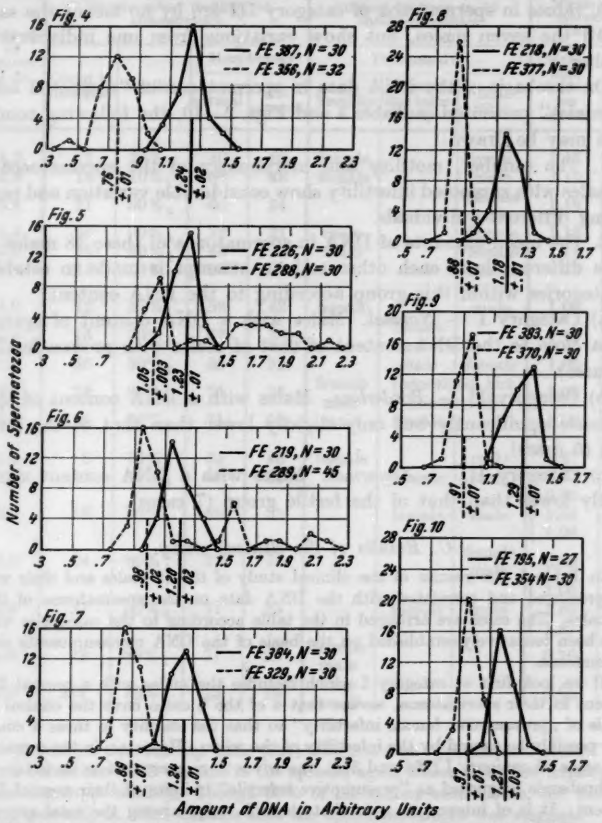
Fig. 4
FE 387, N = 30
FE 356, N = 32
Fig. 5
FE 226, N = 30
FE 288, N = 30
Fig. 6
FE 219, N = 30
FE 289, N = 45
Fig. 7
FE 384, N = 30
FE 329, N = 30
Fig. 8
FE 218, N = 30
FE 377, N = 30
Fig. 9
FE 383, N = 30
FE 370, N = 30
Fig. 10
FE 195, N = 27
FE 354 N = 30
Number of Spermatozoa
Amount of DNA in Arbitrary Units

Figs. 4 to 10. Amount of DNA in spermatozoa of males with suspected infertility (----) and proven fertility (———). Each figure represents an arbitrarily chosen pair of cases (case numbers in upper right corner). Values at vertical lines give mean amounts of DNA. Note that mean values for the cases of suspected infertility in Figs. 5 and 6 do not take into account the DNA values of the second and third peaks as represented in Table 3.

lapping of the DNA values of category III with those of the fertile group.

For a better illustration of the deviation in DNA content among spermatozoa of category III, the measurements of all sperms made in

each individual case are presented in a series of comparisons with individual cases from the fertile group chosen at random (Figs. 4, 5, 6, 7, 8, 9 and 10). As can be seen from these figures, the deviations of the DNA values in spermatozoa of category III are by no means the same for all the seven males, but show variations from one individual to another.

On the basis of the DNA data in spermatozoa of "suspected infertile males" presented in Table 3 and Figs. 2—10, the following conclusions may be drawn:

1. The number, motility and morphology of the spermatozoa of 18 males with suspected infertility show considerable variation and range among different individuals.

2. The mean amounts of DNA in spermatozoa of these 18 males are quite different from each other and an attempt is made to establish 3 categories within this group according to the DNA content.

a) Category I — *Normal.* Males with a DNA content of spermatozoa close to the DNA content of that of males with proven fertility (6 cases).

b) Category II — *Borderline.* Males with a DNA content of spermatozoa significantly but only slightly lower than that of the fertile men (5 cases).

c) Category III — *Abnormal.* Males with a DNA content significantly lower than that of the fertile group (7 cases).

C. Results of the clinical study.

In Table 4, the results of the clinical study of the 18 males and their wives are presented and correlated with the DNA data on the spermatozoa of these 18 males. The cases are arranged in the table according to the categories which have been tentatively established on the basis of the DNA menasurements in the spermatozoa.

If we look first at category I which includes the males with a normal DNA content in their spermatozoa, we see that 4 of the 6 cases have the clinical diagnosis of „presumptive female infertility" so that the sterility in these 4 couples may possibly be caused by the infertility of the wives. However, in the remaining two cases of category I (294 and 345), the wives were presumed to be fertile and the husbands diagnosed as "presumptive infertile" in spite of their normal DNA content. It is of interest to note that these 2 males among the total group of 18 are the ones who showed the most marked impairment of motility of their spermatozoa. The motility rating for these two cases is, respectively $30\%_2$ and $10\%_1$; in other words, besides having a very small number of motile spermatozoa, the degree of motility was extremely poor in both instances. This observation may indicate that the inadequacy of some spermatozoa lies in factors other than the amount of DNA.

Coming now to Category II, characterized by spermatozoa with DNA content intermediate between the normal and abnormal amounts, it can be seen that only one of the five cases was diagnosed as indicating male infertility, while the other 4 were clinically characterized as borderline male infertility or male fertility.

Table 4. *Correlation between clinical findings, microscopical findings and amounts of DNA in human spermatozoa of 18 cases with suspected infertility.*

Fertility No.	Volume of semen in cc.	Microscopical findings of spermatozoa				Clinical findings in 18 childless couples			Microspectrophotometric findings in spermatozoa	
		Count in millions	Motility	Morphology		Presumptive			Mean amt. of DNA per sperm	Categories established on basis of DNA measurements
				normal	abnormal	Fertility	Borderline infertility	Infertility		
294	4.2	7	30% 2	40	60	female		male	1.17	
345	5.8	18	10% 1	52	48	female		male	1.15	
304	3.4	21	50% 4	66	34			female male	1.18	I Normal
363	4.4	58	90% 4	79	21	male		female	1.12	
358	5.2	78	60% 3	48	52	male		female	1.14	
364	1.8	114	50% 3	80	20		male	female	1.19	
324	4,0	12	50% 1	58	42	female		male	1.08	
307	2.1	15	70% 4	62	38		female male		1.10	II Borderline Normal?
326										
315	2.6	56	80% 4	68	32		male	female	1.08	
366	3.6	72	85% 4	75	25	female male	(conceived but miscarried)		1.02	
374	5.4	166	90% 4	71	29	male	female		1.00	
354	2.6	8	70% 3	55	45	female		male	0.97	
370	3.0	12	70% 3	69	31		female	male	0.91	
289	3.8	16	50% 2	65	35		female	male	0.98 1.54 1.98	
329	3.6	24	80% 4	72	28			female male	0.89	III Abnormal
288	4.4	27	50% 3	77	23		female	male	1.05 1.60 1.87	
356	3.8	78	80% 4	71	29	female male			0.76	
377	5.2	231	70% 3	75	25	male female			0.88	

It is of interest that the one male (324), diagnosed as "presumptive sterile" and with a borderline DNA content in the sperms, again shows a marked impairment of the degree of motility. Though 50% of his sperms were motile, the motility rating was only 1. This denotes that the sperm movement was sluggish.

Coming now to category III, that is the seven males who all had amounts of DNA in their spermatozoa significantly different from the normal content, it can be noted that five of the seven husbands were diagnosed as "presumptive sterile" whereas two of the males (356 and 377) as well as their wives, were classified as "presumptive fertile", though sterility existed. Since these two couples are of particular interest, their history is given here in somewhat more detail. No. 356, who had a mean amount of DNA of only 0.76 in his spermatozoa, is a 25 year old colored man who is normal according to all criteria employed in the clinical investigation. The semen analysis is also normal according to the accepted standards; showing normal volume, good count, high motility and good morphology.

His wife had a negative Hühner test on two occasions and, although there was a slight endocervitis, this was not considered sufficient in degree to render the test negative; she was presumed to be fertile. No. 377, a man who also had a low amount of DNA in his spermatozoa, (0.88) is a 37 year old white man who smokes to excess but is otherwise judged clinically normal. The quality of his semen is classified as excellent according to the accepted standards. Here again, as in the previous case, the Hühner test was twice negative for the couple, but his wife was presumed to be fertile.

Though it is remarkable that in both these cases, the low DNA content in the spermatozoa is the only criterion of abnormality observed in the semen, this does not necessarily indicate that the sterility of these two couples is caused by this deviation in the DNA content. Caution for such an interpretation is especially needed since the diagnosis of "presumptive female fertility" is far from absolute.

Table 5. *Correlation between clinical diagnosis of 18 human sterile couples and amounts of DNA found in spermatozoa of male partner.*

No. of couples	Clinical diagnosis	Amount of DNA in spermatozoa		
		normal	borderline ?	abnormal
7	Male infertility Female fertility	2*	1	4
5	Male fertility Female infertility	3	2	
3	Male fertility Female fertility (sterility unexplained)		1	2
3	Male infertility Female infertility	1*	1	1

* Marked impairment of motility of spermatozoa.

On the basis of the individual clinical data of the 18 sterile couples, four groups can be established as has been done in Table 5. If one attempts to correlate the different groups obtained by clinical diagnosis with the DNA measurements in the spermatozoa, the following conclusions can be drawn:

1. From 10 males clinically diagnosed as infertile, 5 had an abnormal DNA content, 2 a borderline DNA content, and 3 a normal DNA content, though the last three show a marked impairment of the motility of the spermatozoa.

2. Of the 8 males clinically diagnosed as fertile, 3 had a normal DNA content, 3 had a borderline DNA content, and 2 had an abnormal DNA content.

It thus appears that the *clinical diagnosis* of fertility or infertility in the male partners of a sterile couple is not necessarily associated in each case with normal and abnormal amounts of DNA in spermatozoa, respectively.

IV. Discussion of the Results.

The results of the DNA measurements in spermatozoa of human males will be discussed, first in their bearing on the problem of fertility, and secondly in relation to the cytological and cytochemical issues involved.

A. DNA content of human spermatozoa in relation to the fertility problem.

While the study of human infertility has made great strides in the last 15 years (for review papers, see ENGLE 1946; NELSON and MAC LEOD 1952; MACLEOD 1951), leading to the recognition and better understanding of some of the complex factors involved, there nevertheless remain many unsolved questions which are particularly apparent when the clinician attempts the diagnosis of female or male infertility in a sterile couple. Due to the multiplicity of factors (hormonal, anatomical, functional) which may cause infertility and the lack of *specific* tests proving infertility, the diagnosis must necessarily be on a *presumptive basis* in most cases. Analysis is handicapped by the fact that experiments designed to test the fertility and fertilizing capacity of the partners, such as constitute the natural and fruitful approach to problems of sterility in plants and other animals, are obviously impossible in the human.

In view of the uncertainty of the diagnoses of presumptive fertility and infertility in the human, any attempt to correlate the DNA data of spermatozoa with fertility or infertility of the male partner of a sterile couple must therefore be approached with the utmost caution. Admittedly it is tempting to postulate that the sterility of some of the couples under examination is due to abnormal amounts of DNA in the sperms. But in view of the lack of any positive tests for female infertility on the one hand and, on the other, the impossibility of testing directly the fertilizing capacity of the suspected spermatozoa in females of proven fertility, such a conclusion must necessarily be considered as premature. It is, however, clear from our results that the possible implication of the DNA of the spermatozoa in the problem of male infertility demands further study. Obviously such exploratory work can best be pursued in mammals other than the human, where direct experimentation and an analysis of spermatogenesis are possible.

B. Cytology and Cytochemistry.

From the cytological as well as the cytochemical point of view, the most striking feature of this study lies in the quantitative differences of DNA that exist between the sperms derived from males of proven fertility and those of the "suspected sterile" group. In the evaluation of these differences it is well to note the remarkable uniformity that obtains in the normal sperms of fertile males. The mean value of the DNA in the 563 sperms measured (1.22) is about half of the mean value obtained in 120 skin cells (2.66), and this is what might be expected in comparing haploid with diploid cells. But whereas the scatter of measurements in the sperms is exceptionally small, the values found in the skin cells have a disproportionately great range (Fig. 11). This, to some

extent, reflects the uniformity of functional germ cells in general and also confirms the recent findings of Tanaka (1951) and Hsu (1952) who found deviations from the normal number of chromosomes very common in some mammalian somatic tissues.

At present only two methods are available for the measurement of DNA in individual cells. Both involve microspectrophotometric techniques, but whereas one employs the absorption of ultraviolet light by

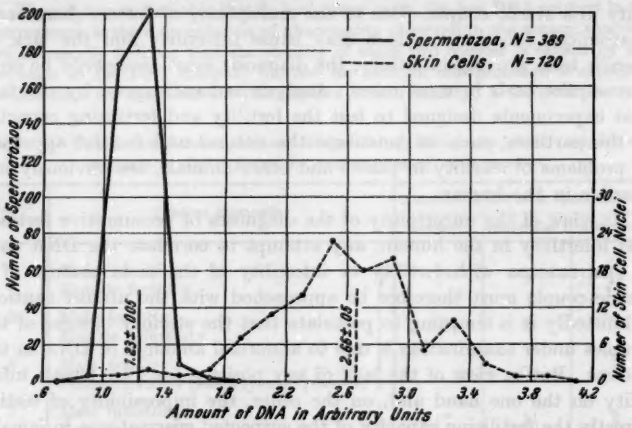

Fig. 11. Comparison of the amount of DNA in individual spermatozoa of fertile males and of individual normal human skin cells. Mean DNA values at vertical lines.

unstained cells (Caspersson 1936), the other involves a measurement of the visible light that is absorbed by specifically stained cell structures (Pollister and Ris 1947). It is not to be denied that a certain amount of error is inherent in both techniques, though many improvements have been made since they were first employed. But that they are sufficiently accurate to give satisfactory answers to many of our problems is attested by the fact that both methods give results that correspond very well to the biochemical assays whenever the latter are available (Leuchtenberger, Vendrely and Vendrely, 1951; Leuchtenberger, Leuchtenberger, Vendrely and Vendrely 1952; Ris and Mirsky 1949). Further support for the accuracy of the photometric methods is encountered in the excellent correlation between the varying chromosome numbers of the cells in ascites tumors (Hauschka und Levan 1951) and photometric measurements of the DNA contents of such cells, made quite independently by Leuchtenberger, Klein and Klein 1952).

This would seem to provide evidence that variations in chromosome number may be recognized by our present photometric methods, as long as sufficient consideration is given to individual departures from mean values. The danger in relying too much on mean values alone is shown by our measurements of the DNA in skin nuclei (LEUCHTENBERGER and LUND 1952; LUND, LEUCHTENBERGER and GOLDSTEIN 1953). The mean of the latter corresponds very nicely to what one would expect after determining the DNA in the haploid sperms, but it actually masks an extraordinary amount of variation in the DNA contents of individual skin nuclei.

In the face of this evidence the small scatter shown by the DNA values of normal sperms cannot fail to carry some assurance that this uniformity is biologically significant. All the more striking therefore, are the data obtained on sperms from the "suspected sterile" males. As mentioned previously, only sperms with a homogeneous distribution of DNA and showing no morphological abnormality were selected for measurement. Six of these males had sperms with a DNA content close to — but not exceeding — that of the sperms of males with proven fertility. But in the remaining twelve males of this group, the mean DNA fell distinctly below the normal amount. However, it should be noted that two of these cases (Figs. 5 and 6) carried a considerable number of sperms with abnormally high quantities of DNA. In short, though the deviation in the DNA of this group of males is preponderantly in a minus direction, a consideration of individual cases suggests that more than one factor is involved.

The fact that in two-thirds of the „suspected sterile" group the sperms carry an amount of DNA that differs from the normal amount is in itself of interest as correlated with at least one type of infertility. But a more detailed consideration of these results makes possible a further step in the analysis. As already mentioned, mitotic irregularities, such as nondisjunction, are common in some somatic tissues. They would be expected to give rise to cells which lack some chromosomes of the diploid set but also to cells that carry a correspondingly greater number. This expectation was realized in the counts made by TANAKA (1951) in various rat tissues, though it should be noted that he found considerably more cells on the plus side than on the minus side (54:34). In contradistinction, HSU's counts (1952) of chromosomes in human spleen cells from tissue cultures showed distinctly fewer cells with chromosomes that exceeded the diploid count than fell below it (4:28). HSU suggested that some chromosomes are occasionally shunted out of the spleen cells, thus trying to account for the predominance of "hypo-aneuploid" cells.

Though Hsu's suggestion is obviously in need of further support, and though Tanaka's findings are rather against such an interpretation, it must nevertheless be held open as a possible factor in our DNA measurements of the sperms of "suspected sterile" males. On such a basis, some of the variation may be accredited to ordinary chromosomal non-disjunction and this might explain the exceptional cases shown in Figs. 5 and 6. But on this same basis most of it would be due to the complete elimination of whole chromosomes from the cell, resulting in DNA deficiency such as is encountered in the remaining ten cases. In short, this type of sterility is due to conditions in the testis which permit a greater irregularity in chromosome distribution than is encountered in the spermatogenesis of fertile males.

But since an elimination of chromosomes from the cell is ordinarily very rare, a second hypothesis must also be considered. According to this, some defect in the process of its synthesis is responsible for the deficiency of DNA in the sperms; or else DNA is broken down or lost from the sperm after having been synthesized. Defects of synthesis must express themselves long before the sperms take their final shape, since all available evidence indicates that no more DNA is formed after the meiotic divisions have begun. On the other hand, losses or a breakdown of DNA could conceivably occur at stages as late as the final shaping of the sperm.

It is clear that a further and more exact analysis of such losses or synthetic defects would have to concern itself with the role played by the DNA in the structure and division of chromosomes.

Summary.

Microspectrophotometric studies were made on the amounts of DNA in individual spermatozoa of 21 human males with proven fertility and on 18 human males who are partners in sterile couples. The results were as follows:

1. The amount of DNA per spermatozoon of the 21 fertile males is very constant and uniform within each individual and among the 21 different individuals. The mean amount of DNA in arbitrary units is 1.22 ± 0.005 per sperm and is approximately one-half that of human somatic cells which is 2.66 ± 0.05.

2. In contrast to the uniformity of DNA in the sperms of fertile males, the sperms from the 18 males under examination for "suspected sterility" showed greatly varying amounts of DNA.

3. 6 of this suspected group had DNA values close to, but not exceeding that of the fertile males. But for 4 of these 6 males this normal DNA amount conformed to the clinical diagnosis that not the males, but their female partners were responsible for the infertility. The

remaining 2 males had sperms with impaired motility, a manifestation which may involve a different factor than that of deviations in the amount of DNA.

4. 5 males represented a category of borderline cases in which the mean DNA was slightly lower than that of the normal group, but many individual measurements overlapped those of normal sperms. So far as the clinical diagnosis was concerned, 4 of these 5 males were on the borderline for male fertility while 1 male had been diagnosed as infertile because he had sperms with impaired motility.

5. 7 of the suspected group carried sperms with DNA in decidedly lower amounts than those of normal, fertile sperms. In 5 of these 7 males the clinical diagnosis ascribed the infertility to the males. In the remaining 2 cases where the DNA was also found to be low in the spermatozoa, no cause could be given clinically for the sterility either in the male or in the female partner.

6. These data suggest a correlation between a deficiency of DNA in the sperms and one type of male sterility, but this cannot be considered as established until further data (such as more information on the female partner) become available.

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Chromosoma, Bd. 6, S. 79—90 (1953).

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SUPPLEMENTARY NOTES ON THE CYTO-TAXONOMY OF MANTIDS (ORTHOPTERA: MANTOIDEA).

By

SALLY HUGHES-SCHRADER.

With 23 figures in the text.

(Eingegangen am 20. Juli 1953.)

This report is a supplement to my review (HUGHES-SCHRADER 1950) of the chromosomes of mantids in relation to taxonomy, and provides further evidence of the need for a critical revision of parts of the classification now in use. Included are data on seven hitherto unstudied species, representative of five subfamilies. One of these, the *Miopteriginae*, has not previously been sampled cytologically; and for two others, the *Oligonicinae* and the *Epaphroditinae*, data on male meiosis have heretofore been lacking. Cytological observations on each species are presented below under the subfamily categories of GIGLIO-TOS (1927), to facilitate comparison with my earlier report. The concluding section reviews the cytological evidence in relation to taxonomy.

Material and Methods.

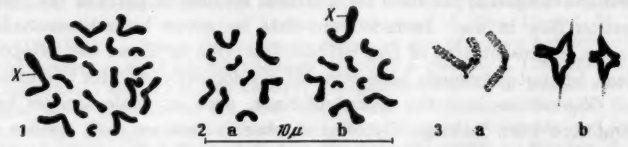
Six of the species studied were collected in Trinidad, B.W.I.; these are: *Promiopteryx granadensis* (SAUSSURE), *Thesprotia filum* (LICHTENSTEIN), *Tithrone roseipennis* (SAUSSURE), *Acontiothespis multicolor* (SAUSSURE), *Acanthops falcata* STOL, and *Oxyopsis rubicunda* (STOL). Field notes and photographs of dried specimen of each of the foregoing are available in BEEBE, CRANE and HUGHES-SCHRADER (1952). The seventh species, *Thesprotia graminis* (SCUDDER), was collected in Florida, USA, during March, 1952. It was abundant throughout the state, and in a variety of habitats, as the following records of the six males used for cytological study indicate: March 18, an adult male was beaten from Spanish moss dependent from water oak, lakeside near Inverness; March 20, a juvenile male was taken by sweep net from grassy meadow near Bradenton; March 21, one juvenile and one adult male were swept from low ground cover in pine woods near Fort Myers; and, March 26, one juvenile and one adult male were swept from dense roadside thickets on Upper Matacumbe Key.

The material comprises testes from adult and juvenile males, supplemented when possible with ovaries from juvenile females. Fixation was in acetic acid-absolute alcohol (1 : 3), selected for its suitability for microspectrophotometry for which part of the material was destined. In comparing chromosomes figured here-in with those illustrated in my 1950 review, allowance should be made for the greater contraction induced by Carnoy as compared to Sanfelice fixation. All material was sectioned, and stained by the Feulgen method, followed when desirable by a counter stain of Fast Green. All drawings were made with Abbe camera lucida at table level, Zeiss apochrom. obj. 90, oc. 20; the magnification is uniform throughout and is indicated by scale on the figures.

Observations:

Subfamily *Miopteriginae*.

Promiopteryx granadensis is the first representative of this exclusively neo-tropical group to be studied cytologically. Its most striking cytological characteristic is the small size of its chromosomes—both absolutely, for they range in length from 6.5 to 2 microns, and relatively to those of most other mantids. Among the species thus far studied, only *Pseudomiopteryx infuscata* parallels it in this respect; the total length of all the chromosomes of the complement is approximately the same in these two karyotypes, and does not exceed half that characteristic of such manteine genera as *Stagmomantis* and *Tauromantis*. And since chromosomal diameter is roughly proportional to length in the two groups, total



Figs. 1—3. *Promiopteryx granadensis*, ♂. — Fig. 1. Gonial metaphase. — Fig. 2. Meiotic metaphase II; *a* with no X, *b* with X. — Fig. 3. Non-chiasmate bivalents; *a* at prometaphase, *b* at metaphase I.

length gives an approximate index of total chromosomal mass. The chromosomes (2n ♂), all mediokinetic, number 19 (Fig. 1). The sex chromosome mechanism is of the XO—XX type. The X, while the longest of the set, exceeds only slightly in length the longest of the autosomes (Fig. 2).

Again as in *Pseudomiopteryx*, spermatogenesis occurs unusually early in the life cycle. My material, abundant on late meiotic stages, is scanty on the actual divisions; but it suffices to establish the following characters in male meiosis: (1) The parallel pairing of homologous chromosomes persists until first anaphase, except for a slight separation of the kinetochore regions at prometaphase and metaphase (Fig. 3). (2) The structure of the meiotic bivalents is non-chiasmate (Fig. 3a and b). When the homologues finally separate at first anaphase no crossing of chromatids is disclosed. (3) The pre-metaphase stretch stage is absent. Possibly a more extensive material should be examined fully to establish this point; however, the close seriation of stages possible in the material at hand, and the precise parallel presented to the course of events in the thoroughly documented cases of *Pseudomiopteryx* and *Callimantis*, leave small room for question.

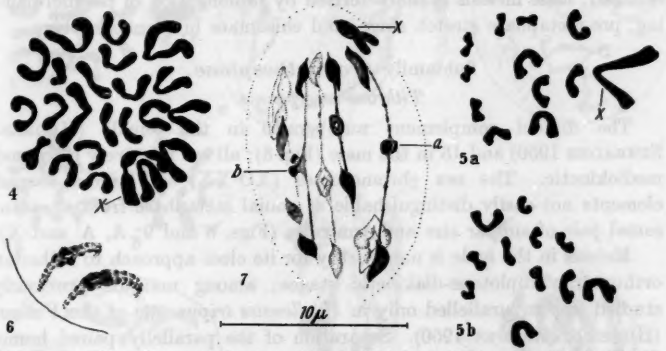
The cytological characters of the subfamily *Miopteriginae* may thus tentatively be summarized as follows: low number and small size of chromosomes; retention of primitive sex chromosome mechanism; and in

male meiosis, the pronounced prolongation of parallel pairing, nonchiasmate bivalent structure, and absence of the pre-metaphase stretch stage.

Subfamily *Oligonicinae.*

Thesprotia graminis of Florida and *T. filum* of Trinidad provide the first data on male meiosis in this group. No cytological differences are apparent between the two species and the following account, although illustrated exclusively from *T. graminis* is valid also for *T. filum.*

The chromosomes, all mediokinetic, number 23 (2 n ♂) and are relatively large (Fig. 4). The unpaired X, the largest of the set, appears



Figs. 4—7. *Thesprotia graminis*, ♂. — Fig. 4. Somatic metaphase. — Fig. 5. Meiotic metaphase II; *a* with X, *b* with no X. — Fig. 6. Two bivalents in late meiotic prophase, just prior to stretch stage, showing prolongation of parallel pairing. — Fig. 7. Pre-metaphase stretch stage; open cross configuration in bivalents *a* and *b*; not all bivalents are drawn.

twice as long as the longest autosome at the second meiotic metaphase (Fig. 5), but in gonial and first meiotic mitoses the difference is slighter. The secondary constrictions in the X of the somatic plate shown in figure 4 are variable in expression and have not been observed in either gonial or meiotic mitoses.

Meiotic prophase in the male shows the bouquet configuration of chromosomes—at first with a single center, and later shifting to a bipolar orientation—which is so characteristic of mantids. Homologous chromosomes remain parallelly paired (Fig. 6) until the spindle forms. At this point a pre-metaphase stretch movement opens the bivalents so violently that the regions between kinetochores and chiasmata or the terminal association of chromosome arms are often attenuated into thin threads. However, wherever analyzable, bivalent structure proves to be chiasmate, as is shown by the open cross formations and alternating planes of opening out visible in bivalents such as *a* and *b* in figure 7.

Congression at the equator without shift in orientation, and recontraction into typical rod and ring bivalents, follow the stretch stage.

Oligonyx dohrnianus, the only other oligonicine for which any cytological data are available, is also an XO-XX type, but with a diploid male complement of 19 (HUGHES-SCHRADER 1950). Comparison of the karyotypes of *Oligonyx* and *Thesprotia* suggests a close relationship and approximate equivalence in total chromosomal mass. The cytological characters of the subfamily as now known may be summarized as follows: chromosome number is in the low to middle range, with total chromosomal mass relatively high; the primitive sex chromosome mechanism is retained; male meiosis is characterized by prolongation of parallel pairing, pre-metaphase stretch stage, and chiasmate bivalent structure.

Subfamily *Acontiothespinae*.

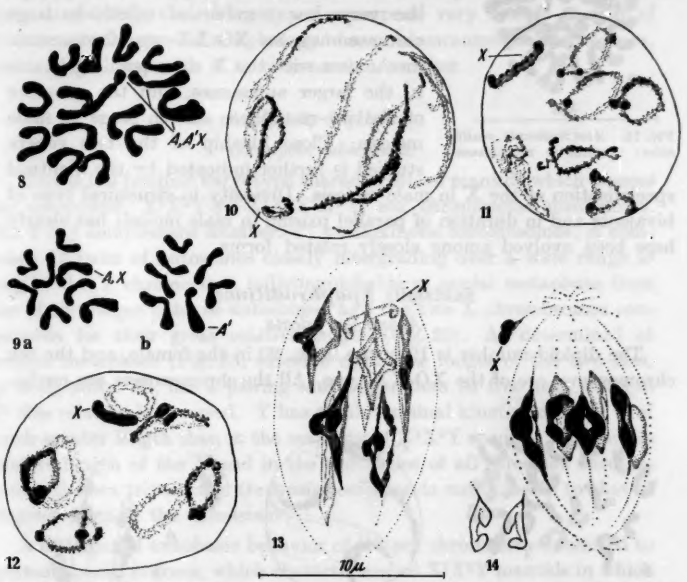
Tithrone roseipennis.

The diploid complement numbers 16 in the female (HUGHES-SCHRADER 1950) and 15 in the male (Fig. 8); all are relatively large and mediokinetic. The sex chromosomes (XO-XX) are large J-shaped elements not easily distinguishable at gonial metaphase from an autosomal pair of similar size and arm-ratio (Figs. 8 and 9; A, A′ and X).

Meiosis in the male is noteworthy for its close approach to orthodox orthopteran diplotene-diakinetic stages; among mantids previously studied this is parallelled only in *Phyllovates tripunctata* of the *Vatinae* (HUGHES-SCHRADER 1950). Separation of the parallelly paired homologues begins at the kinetochore region while the bivalents are still polarized in a double bouquet (Fig. 10). Two or three loci of opening out develop and the resultant loops and half loops lie in alternate planes (Figs. 11 and 12). This latter fact plus the clear open cross formations attest the chiasmate structure of the bivalents. Of special interest is the free spacing of the diakinetic bivalents throughout the nucleus, in contrast to the close apposition of chromosomes to nuclear membrane which so frequently characterizes this stage. A pre-metaphase stretch of only moderate intensity (Fig. 13) is initiated as the spindle forms. Recontraction and congression in a typical metaphase orientation follow (Fig. 14). There is some evidence for a kinetochore-center interaction prior to congression in the second meiotic division also, but the Carnoy fixation of these stages proved inadequate for a positive demonstration.

Two peculiarities of the X chromosome of *Tithrone* merit mention. During first meiotic metaphase the distal third of the long arm of the X is sharply reflexed (Fig. 14, lower left). This often simulates a secondary kinetochore, but no chromosomal fiber is demonstrable. In the ensuing anaphase the X remains quiescent until the autosomes are two thirds of the way to the poles. Belatedly its chromosomal fiber then shortens, and

the X is included as a pendant vesicle in the telophase nucleus. In the related genus *Acontiothespis* (HUGHES-SCHRADER 1950) the X chromosome develops a similar sharp bend at a subterminal locus in the long arm at first meiotic metaphase; in *Acontiothespis*, however, although a differential anaphasic movement of X is probable, too few anaphases were available for scoring to permit a definite demonstration. That the



Figs. 8—14. *Tithrone roscipennis*, ♂. — Fig. 8. Gonial metaphase, X indistinguishable from autosomal pair A, A¹. — Fig. 9. Meiotic metaphase II; *a* with X, again indistinguishable from autosome A, *b* with no X. — Figs. 10—12 progressive opening out of bivalents at diplotene-diakinesis; note alternate planes of opening out, and open cross configurations (two bivalents at lower left. — Fig. 11. not analyzable). — Fig. 13. A group of bivalents and the X during the pre-metaphase stretch. — Fig. 14. Meiotic metaphase I; at lower left the X chromosome from two neighboring cells, oriented to show reflexed distal end of long arm.

two phenomena are not, however, necessarily related is indicated by the occurrence in the eremiaphiline *Humbertiella* (HUGHES-SCHRADER 1948) of differential anaphasic movement of the X in the absence of any structural modification.

Acontiothespis multicolor.

The available material of this species permits only the determination, in somatic plates, of the 2 n ♀ complement of 16 chromosomes (Fig. 15).

Close correspondence in number and morphology is evident with *A. cordillera vitrea* and *Tithrone roseipennis*; the two pairs of large chromosomes with subterminal kinetochores (Fig. 15) correspond with the X chromosomes and the pair of large J-shaped autosomes found in the two last named species.

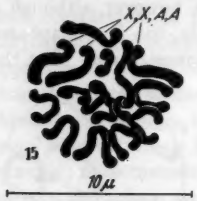


Fig. 15. *Aconiothespis multicolor:* ♀ somatic metaphase.

As tentative subfamily characters appear the very low number of relatively large chromosomes, an XO-XX sex chromosome mechanism with the X corresponding in size to the larger autosomes, and the presence of the pre-metaphase stretch stage in male meiosis. Close kinship of the two genera studied is further indicated by the identical specialization of the X in male meiosis. Diversity in structural type of bivalents and in duration of parallel pairing in male meiosis has clearly here been evolved among closely related forms.

Subfamily *Epaphroditinae*.

Acanthops falcata.

The diploid number is 19 in the male, 20 in the female, and the sex chromosomes are of the XO-XX type. All the chromosomes are medio-

Figs. 16—19. *Acanthops falcata*, ♂. — Fig. 16. Gonial metaphase. — Fig. 17. Bipolar bouquet stage of late meiotic prophase; no further opening out occurs prior to stretch (not all bivalents are drawn in this and the following figure). — Fig. 18. Pre-metaphase stretch stage; open cross configuration in 4 bivalents. — Fig. 19. Autosomal bivalents at metaphase I, following post-stretch contraction and congression.

kinetic and show considerable range in length, with the X by far the largest of the set (Fig. 16).

Male meiosis conforms so closely to the pattern described for *Thesprotia* that only a summary need be given. Homologous chromosomes remain parallelly paired (Fig. 17) until opened by a typical pre-

metaphase stretch movement (Fig. 18). Chiasmata are demonstrable in most of the bivalents during the stretch stage, and are completely terminalized by metaphase (Fig. 19).

These are the first data on male meiosis in the *Epaphroditinae*. The gonial chromosomes, however, are known in two other members of the group—*Acanthops godmani* and *Pseudacanthops medusa* (HUGHES-SCHRADER 1950); their karyotypes correspond very closely to that of *A. falcata* in the general morphology of sex chromosomes and autosomes, and are identical with it in chromosome number.

Subfamily *Vatinae*.

Oxyopsis rubicunda.

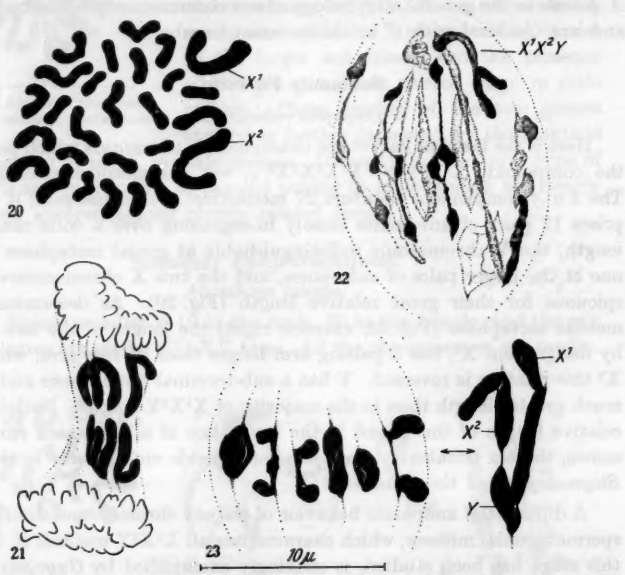
Here is the familiar karyotype characteristic of mantids which possess the compound, X^1X^2Y ♂—$X^1X^1X^2X^2$ ♀, sex chromosome mechanism. The 2 n ♂ complement numbers 27 mediokinetic chromosomes; it comprises 12 pairs of autosomes closely intergrading over a wide range in length, the Y chromosome indistinguishable at gonial metaphase from one of the larger pairs of autosomes, and the two X chromosomes conspicuous for their great relative length (Fig. 20). As determined at meiotic metaphase (Fig. 23, extreme right) the longer of the two Xes, by designation X^1, has a pairing arm longer than its free arm, while in X^2 this relation is reversed. Y has a sub-terminal kinetochore and is of much greater length than in the majority of X^1X^2Y species. Both in the relative length of the Y and in the arm-ratios of all three sex chromosomes, the sex trivalent of *Oxyopsis* corresponds most closely to that of *Stagmatoptera* of the *Manteinae*.

A differential anaphasic behavior of the sex chromosomes, limited to spermatagonial mitoses, which characterizes all X^1X^2Y mantids in which this stage has been studied, is strikingly exemplified by *Oxyopsis* also. Sex chromosomes and autosomes begin the anaphasic movement synchronously but the sex chromosomes are halted midway while the autosomes continue past them on their way to the poles (Fig. 21). Only later, when the autosomes are massed at the poles does there occur a further shortening of the chromosomal fibers of the sex chromosomes bringing them into contact with the distal ends of the autosomes and ensuring their inclusion in the telophase nuclei.

In male meiosis parallel pairing of the autosomes is prolonged until abrogated by the onset of an intensive pre-metaphase stretch stage. The sex trivalent, however, again as in all other X^1X^2Y species in which its behavior has been checked, opens into a chain formation prior to the pre-metaphase stretch and without disclosing any chiasmata. The bivalents, wherever analyzable during the stretch stage, show a chiasmate

structure (Fig. 22), and an occasional open cross is still unterminalized at metaphase (Fig. 23).

In all cytological characters *Oxyopsis* thus corresponds closely with the neo-tropical vatine genera previously studied and would appear to be most naturally associated with *Stagmatoptera* of the *Manteinae*. The significance of these findings for the taxonomic placement of *Oxyopsis* is considered in the discussion.



Figs. 20—23. *Oxyopsis rubicunda*, ♂. — Fig. 20. Gonial metaphase; the long arm of X^1 is fore-shortened. — Fig. 21. Differential movement of sex chromosomes (shown in black) in gonial anaphase. — Fig. 22. Pre-metaphase stretch; alternate planes of opening out and open cross visible in several bivalents. — Fig. 23. Group of bivalents and the sex trivalent (shifted to right for clarity) at metaphase I.

Discussion.

The cytological data on these seven additional species in large measure confirm the conclusions relative to the taxonomic value of the cytological characters of mantids presented in my 1950 review. Prolongation of parallel pairing in male meiosis was there shown to be a highly variable character; indeed its two extremes are encountered in different geographic populations of the same species. Thus the wide divergence with respect to this character between *Tithrone* and *Acontiothespis*, and again between *Oxyopsis* and *Phyllovates*, in no way

gainsays the large body of evidence, based on a comparison of karyotypes and on external morphology, which demonstrates the close kinship of the members of each of these pairs of genera.

The same holds true for the evolution of non-chiasmate bivalents in *Acontiothespis* while, in contrast, *Tithrone* retains the chiasmate structure which was in all probability characteristic of the ancestral mantid stock. As I have shown previously, non-chiasmate meiosis has little or no value as a cyto-taxonomic criterion in mantids: there is good evidence for its multiple origin within the group; indubitably closely related forms vary with respect to the presence or absence of chiasmata, and in some species both types of bivalent structure are found within the same complement. This is not surprising since the prolongation of parallel pairing in male meiotic prophase—a widely spread character of early origin in the mantid stock—provides a mechanism alternative to chiasmata for ensuring the continued conjunction of homologous chromosomes through late meiotic prophase, and should thus have facilitated the evolution of non-chiasmate bivalent structure. The contrary assumption—that the establishment of non-chiasmatic meiosis must represent an extremely rare evolutionary event in any group and hence be indicative of the common descent of those species exhibiting it—rests on the prior assumption that chiasmata can be universally equated with genetic crossing-over, the suppression of which would have serious evolutionary consequences. Since chiasmata have been repeatedly shown to give no reliable index of crossing-over (compare COOPER 1945, 1949), this point of view merits no further comment, especially with reference to a group such as the mantids in which genetic evidence is non-existant.

That the pre-metaphase stretch may prove of cyto-taxonomic value is suggested by the fact that in none of the groups yet studied is there found any such variability in respect to it as is shown by the two characters just discussed. On the contrary, in the only natural group of which a fairly large sample of species can be scored, there is complete agreement with respect to this character. This group comprises the X^1X^2Y mantids; here in all 10 species, including *Oxyopsis*, in which the course of male meiosis is known, the pre-metaphase stretch stage is present.

Promiopteryx is of interest in providing the first cytological data on the *Miopteriginae*; so close is the correspondence with the *Pseudomiopteriginae* as represented by *Pseudomiopteryx* that cytologically no basis for separation of the subfamilies is apparent. Most significant of their common attributes is the low number and small size of the chromosomes which in total mass do not exceed onehalf that characteristic of *Stagmomantis* and other X^1X^2Y species. The relative amount of DNA (desoxyribose nucleic acid, microspectrophotometrically determined) in the nuclei of *Pseudomiopteryx* as compared with *Stagmomantis* confirms

this ratio. An hypothesis of tetraploidy in the X^1X^2Y mantids based on these data, and in certain XO *Empusinae* which combine a high chromosome number with an X of extreme length relative to the autosomes, has been previously proposed (HUGHES-SCHRADER 1951), and was independently advanced by GOLDSCHMIDT in a paper only recently made available (GOLDSCHMIDT 1953—sent to press 1949). Theoretically the establishment of polyploidy in mantids appears highly plausible since the prerequisite stabilization of the sex determining mechanism is readily visualizable within the framework of our knowledge of the chromosomes of mantids now living—either through fusion of the two Xes following a doubling of the chromosome number, or through the transformation of the sex chromosomes into a compound mechanism. The first process is inferred from the existence of karyotypes such as those of the *Empusinae* cited above; the second may be visualized as the result of any one of several structural rearrangements occurring either simultaneously with the doubling of the chromosome number or subsequent to it (GOLDSCHMIDT 1953; and, without implication of polyploidy, in WHITE 1941, and OGUMA, cited in HUGHES-SCHRADER 1950). But in spite of its plausibility, and although the ratios in total chromosome length and in nuclear content of DNA between *Pseudomiopteryx* and *Stagmomantis* support it, the evidence for polyploidy is far from conclusive, since one case is known in which a gross change in DNA values has occurred among species of the same genus with no detectable effect on total chromosome mass (HUGHES-SCHRADER 1953). So extensive have been the structural rearrangements and numerical changes in the chromosomes during mantid evolution that suspected polyploidy cannot be tested by the structural homology of individual chromosomes. It is therefore to be hoped that the DNA values in the species involved will give a less ambiguous answer, but obviously much further work is necessary to determine the relative validity of the DNA values as cytotaxonomic criteria.

The compound, X^1X^2Y sex chromosome mechanism has potentially high taxonomic value, for it has been shown to give unusually direct evidence of the descent of those forms now possessing it from a single ancestral species (WHITE 1940, 1941; HUGHES-SCHRADER 1950). The close cytological agreement here reported between *Oxyopsis* and all other X^1X^2Y species thus far studied—agreement extending even to minute details in the uniquely specialized mitotic behavior of the sex chromosomes—provides additional evidence that the mantids characterized by this mechanism form a natural group of directly related genera. In the classifications now in general use the known members of this group are referred to no less than 5 subfamilies (GIGLIO TOS 1927), or to 3 rather widely separated families (CHOPARD 1949). OGUMA (1946)

recognizes the homogeneity of the X^1X^2Y group and his suggestion of a primary subdivision of the *Mantoidea* on the basis of sex chromosome mechanisms should be considered in the critical revision of the higher categories in mantid classification which is so obviously needed.

Oxyopsis is the third genus of the neo-tropical *Vatinae* now shown to be of X^1X^2Y constitution and hence to be almost certainly wrongly associated with the Old World *Vatinae* represented by *Aethalacroa ashmoliana* (OGUMA 1946). The neo-tropical genus *Stagmatoptera* has already been transferred (REHN 1935) from the *Vatinae* to the *Manteinae* on taxonomic grounds, later confirmed cytologically (HUGHES-SCHRADER 1950); the cytological evidence indicates that *Oxyopsis*, *Vates* and *Phyllovates* should follow suit. Significant in this connection is the agreement in behavior patterns between the vatines *Oxyopsis* and *Vates* and the manteines *Stagmatoptera*, *Stagmomantis* and *Parastagmatoptera* (CRANE 1952). CRANEs observation of copulation, in captivity, between an *Oxyopsis* female and a *Stagmatoptera* male points in the same direction (BEEBE, CRANE and HUGHES-SCHRADER 1952).

In conclusion, it is encouraging to note the agreement, already mentioned in the case of *Oxyopsis*, between cytological and behavior studies in their bearing on mantid taxonomy. CRANE (1952), in discussing the phylogenetic significance of innate defensive behavior in mantids, points out that, while broadly speaking the data harmonize with the conclusions derived from comparative morphology, two instances are disclosed in which behavior does not correspond to a designated taxonomic position (BEIERS placement of *Liturgousa* and *Angela* in the *Manteinae*). In both these cases the cytological evidence confirms that of the behavior studies, and supports the more generally accepted placement of these genera. In several areas of mantid systematics an underlying basic relationship has become masked by striking morphological specializations; it is precisely here, in the evaluation of such characters, that evidence from allied fields can be most useful in the development of an evolutionarily significant classification.

Summary.

1. Chromosome number, sex chromosome mechanism and the salient features of male meiosis have been determined for the following neotropical mantids: *Promiopteryx granadensis* (SAUSS.), *Thesprotia filum* (LICHT.), *Thesprotia graminis* (SCUDDER), *Tithrone roseipennis* (SAUSS.), *Acanthops falcata* STOL, *Oxyopsis rubicunda* (STOL); and the female diploid number for *Acontiothespis multicolor* (SAUSS.). This adds one more subfamily of mantids, the *Miopteriginae*, to the list for which cytological information is now available, and gives the first data on meiosis for two others—the *Oligonicinae* and the *Epaphroditinae*.

2. The subfamily *Miopteriginae*, as exemplified by *Promiopteryx*, is characterized by the XO-XX sex mechanism and in male meiosis by prolongation of parallel pairing, non-chiasmate bivalent structure and absence of the pre-metaphase stretch stage; close correspondence with the *Pseudomiopteriginae* is evident. Relatively low number and small size of chromosomes give a total chromosomal content approximately half that found in X^1X^2Y manteine karyotypes. The possibility of a polyploid relation is discussed.

3. *Oxyopsis*, presently assigned to the *Vatinae*, is shown to belong to the natural group of genera characterized by possession of the compound X^1X^2Y sex chromosome mechanism; cytologically it is most closely associated with *Stagmatoptera* of the *Manteinae*.

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